THE SPLENIC CIRCULATION

With Special Reference to the Function of the Spleen Sinus Wall

BY

SVEN ERIK BJÖRKMAN

This work is also published as Supplementum CXCI of Acta Medica Scandinavica

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MED. LIC., GBG.

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FROM THE MEDICAL CLINIC AT THE UNIVERSITY OF UPSALA
CHIEF: PROFESSOR E. ASK-UPMARK
AND
THE PATHOLOGICAL DEPARTMENT AT THE UNIVERSITY
OF UPSALA
CHIEF: PROFESSOR R. FÅHRÆUS

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Preface

The present paper will be a tribute to the solution of the problem of splenic circulation. This question may be regarded as one of the best guided secrets of anatomy since numerous investigations during more than 100 years have not yet succeeded in giving it a satisfactory answer. Tho being mainly a morphologic problem, it has, nevertheless, several physiologic aspects, and may hold the clue to the mystery of the physiologic significance of the spleen.

This investigation was started through an occasional observation made during work on an altogether different subject in 1941. My chief then, Prof. GUSTAF BERGMARK, M. D., has ever since that time encouraged me to proceed with this work, and has, in many ways, enabled me to carry it to its present state. I will take this opportunity of thanking him heartily for all his help and encouragement. I will at the same time express my sincere thanks to his successor as the present chief of the Medical Clinic, Prof. ERIK ASK-UPMARK, M. D., who — being himself an authority on splenic questions — has hurried me on the way of carrying this work to a conclusion.

Working principally at the Pathological Department at the University of Upsala, I have had a never-failing adviser in its head, Prof. ROBIN FÅHRÆUS, M. D., whose pioneering inquiries into the blood and spleen physiology are well known. For his invaluable help and very stimulating suggestions I feel the deepest gratitude, and wish thus to express it.

With regard to the statistical treatment I had the pleasure of receiving advice from Prof. GUNNAR DAHLBERG, M. D., Chief of the Swedish State Institute for Human Genetics and Race Biology, Upsala. Without this help of his I should not have been able to estimate the skewness in the distribution of the animal material. To him I desire to pay due respects. The statistical calculations have, in part, been done by Mrs. MARTHA BUME, at the same Institute. I truly wish to express my appreciation of her valuable work.

Miss VERA STERNER, histo-technical assistant at the Pathological Department, has in a skilful and accurate manner aided me by making the technical laboratory work. I am very thankful for her painstaking work.

The translation into English has been made by Rev. KONRAD HOLM, Th. Ph. Lic., graduated in America, whom I wish to offer my sincere gratitude for his careful rendition.

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Uppsala, April, 1947.

Sven Erik Björkman.

Contents

Introduction 7

I. Review of Literature 9

Earlier Observations 9

The Circulatory Problem 11

The Open Circulation 12

The Closed Circulation 17

The Divided Circulation 23

The Minute Structure of Spleen Sinuses 28

Pathological Anatomy of the Sinus Wall 34

Physiological Considerations 35

The Reservoir Function 35

Qualitative Alterations in the Red Cells during the Reservoir Function 38

PERSONAL INVESTIGATIONS

II. The Problem 42

III. Animal Experiments 44

The Red Corpuscle and the Sinus Wall 44

Injection of Gelatin 45

Injection of Saponin 46

Attempts to Use an Indirect Method of Measuring the Size of the Stomata in the Spleen Sinus Wall 47

Attempts at Estimating the Size of the Stomata in the Sinus Wall in Experimental Infections and Intoxication with Hemolytic Poison 54

IV. The Human Spleen 62

Analysis of Alterations in the Sinus Wall Structure Taking Place during Variations in the Sinusoidal Volume 62

Measurement of the Mutual Intervals between the Annular Fibrils in Human Spleens.....

The Sinus-Sphincter 69

Structural Modifications of The Sinus Filter under Certain Conditions..... 71

Interpretation of the Changes taking Place in the Reti-Layer of the Sinus Wall..... 74

V. Summary and Conclusions..... 79

Bibliography..... 85

Introduction

The spleen is invariably found in all vertebrates except the lancelot. In the animal series it appears simultaneously with the hemoglobin and the enclosure of the coloring matter of the blood in the red corpuscles. The spleen reaches its relatively largest size in the mammals.

It has a characteristic histological anatomy, and is the richest vascularized organ of the body. Besides lymphocytes, the principal portion of the splenic parenchyma consists of reticulum cells. So the spleen represents a part of the Aschoffian reticulo-endothelial system together with the lymphatic glands, the liver, the bone marrow as well as other organs.

Physicians of ancient times attached a great pathogenetic significance to the spleen. There they thought the blood to be cleansed from a surplus of black bile, *bilis atra* or *succus melancholicus*. In a disturbance of this function one would meet with melancholy. The spleen is also called the »organ de rire» in a French encyklopedia of 1765.

Modern physiology has not yet succeeded in explaining the function of the spleen. The method of drawing conclusions from the disturbances occurring at the removal of an organ with respect to its normal function, is here useless, because splenectomy does not involve any constantly reproducable changes. The spleen is, accordingly, not an organ of vital importance. So far as man is concerned, Ask-Upmark proved that in 1935 through an after-examination of one hundred cases, which had been splenectomized on account of traumatic rupture of the spleen; none of them showed any increased morbidity after the operation.

On the other hand, splenectomy has resulted in some well-known therapeutic effects in certain hematological disorders, which indicate that a disturbance in any of the splenic functions may threaten very life. Nor does the pathologist give us any help in solving the riddle of the spleen; for there appear no typical histologic changes at

such splenic disorders. The indications for splenectomy are, therefore, less based upon patho-physiological experiments then upon clinical experience.

The rich supply of vessels and the favorable effect of splenectomy in certain cases have supported the assumption that splenic function should be concerned with the metabolism of the blood; the spleen has principally been regarded as an organ destroying erythrocytes.

One difficulty in trying to make clear the functional connections, arises from the fact that we have not yet been able to bring forth a complete picture of the pathway of the blood from artery to vein in the splenic pulp.

I. Review of Literature

Earlier Observations

As early as 1686, MALPIGHI — who deserves much credit for his accomplishments in the structural investigation of the spleen — observed certain peculiar conditions in regard to the splenic vessels. By inflating spleens with air from the splenic vein and then by drying them, he demonstrated the pathways of the intralienic veins. In their finest ramifications he found the walls perforated. These tiny holes — later called *stigmata Malpighi* — constitute, as we now know, the orifices of the spleen sinuses.[1] He thought them to be communications within the septa which he found separating the thin-walled veins from one another. These septa seem to have been composed of the desiccated red pulp. Thus MALPIGHI was right when he anticipated the occurrence of some special cavities in these septa which had connections with the venous network.

His later contemporary, the Dutch RUYSCH, became known for his ability to produce anatomical preparations through vessel-injections. By means of his experiments he reached the conclusion (1696) that the main bulk of the spleen is made up of vessels. He showed that the arteries have a free course without anastomosing and that they end with a pencil-like dispersion into fine ramifications, which he called *penicillii*. They voided their content into the juicy pulp, where they, according to his opinion, had intercourse with the lymphatics.

The improvement of the microscope as well as new histologic methods gave rise to the appearance of the cellular histology and pathology in the fourth decades of the 19th century. This brought about a whole series of anatomical discoveries, also concerning the

[1] In order to avoid the term 'venous sinus', which implies an altogether wrong idea, viz. that the sinuses should constitute a section of the veins, the more neutral word 'spleen sinus' is here used for this specific formation.

spleen. In a few decades they made clear the particulars of the splenic structure, which for this very organ are of essential importance.

The great physiologist JOHANNES MÜLLER (1834) carried out injection experiments on the splenic vessels in order to find the exact position of the Malpighian corpuscles in relation to the arteries. After injecting mercury or cinnabar, he discovered that the pencil-shaped arteries emptied themselves into what he calls venous anastomosing channels, which he found in the red pulp. They correspond probably to what we now call spleen sinuses. About these channels, which passed over into the veins, he writes: "Sie sind sehr merkwürdig. Diese ziemlich starken anastomosierenden Anfänge der Venen scheinen kaum noch eine Wandung zu haben. — Injicirt man Wachsmasse durch die Venen, so erhält die Milz das Ansehn der Corpora cavernosa penis." The spleens, which MÜLLER examined, came from swine, sheep and cattle.

ALBERT VON KÖLLIKER (1849) was the first one to propound the theory that the spleen destroys red corpuscles through dissolution. He states that the finest walls of the veins consist only of one endothelial layer and describes the cells of that as being rather long and serpiginous in their edges, sometimes rolled up into balls. These must have been sinus endothelial cells; KÖLLIKER held them, however, to be muscular cells belonging to the trabeculae.

KÖLLIKER believes that the blood in the spleen extravasates easier from its vessels than it does in any other organ. Moreover, he foresaw that neither injection experiments nor histologic examination would ever explain the vascular connections in this organ. In conjunction with the observations that led him forward to the conviction that erythrocytes do perish in the spleen, he writes in *Todd's Cyclopedia of Anatomy* as follows: "The spleen is a contractile organ and may, by virtue of its contractility, be able to dilate and contract itself, to fill itself with blood, and again to expel the blood from it. In the state of filling with blood, a stagnation of blood occurs in the smaller vessels, perhaps even an extravasation; and in this stagnant blood the globules undergo destruction, since they dissolve themselves, either free or inclosed in cells." This expressed view of his agrees exceedingly well with that picture of circulation which KNISELY gives us in 1936.

In his book, "The Spleen" (1854), HENRY GRAY gives us a detailed statement about the minute anatomy of the spleen. Through injec-

tion experiments, he thought himself able to point out an anastomosing capillary network in the red pulp. Between the capillaries he saw pulp meshes considerably wider than the capillaries. He believed these spaces to constitute the proper parenchyma of the spleen. The capillaries emptied into veins, which from the very beginning were remarkably wide. Then, he also held that some capillaries empty themselves into the pulp meshes.

The channels which GRAY saw injected in his preparations, seem to have been made up of spaces in the pulp cords, while his pulp meshes must have been spleen sinuses. When he declares that the arterial capillaries empty into veins, he bases this thesis upon his interpretation of those channels as capillaries. This mistake has later been repeated by A. KEY among others. The explanation seems to be this that GRAY's injection bulk was, as a matter of fact, lying freely between the cells of the pulp cords.

As it appears from description above, several writers have early noticed a peculiar channel system in the splenic red pulp. But it was granted to THEODOR BILLROTH (1860), the great German surgeon, to explain, furthermore, the real nature of these channels. He examined materials, derived from man and different animals, emphasizing the significance of an orderly fixation method, as well as an approved histologic technic. He found that the veins in the human spleen originate from a great number of capillary splenic veins, which form a part of the red pulp. He ascertains that all of them are of about the same width, ca. 90—100 micra, and that they on sections look like venous *rete mirabile* or *teleangiektasia*. They could be injected from the vein, but with greater difficulty from the spleen artery. Between these capillary veins he found an intervascular texture, in which he saw pulp meshes, even as wide as 20 micra, containing partly red, partly white blood corpuscles.

The Circulatory Problem

The capillary veins are formations, now called spleen sinuses, altogether characteristic for this organ. By his great discovery BILLROTH created the problem concerning the connections between the *penicillii* and the capillary veins. This brought on a debate that has not yet ended. The methods of investigation consisted of observations in the microscope, estimation of the position of injected matters

and, in recent years, observation of the circulation within the living spleen in experimental animals. At least during the latter half of the 19th century, when the splenic circulatory problem assumed a dominating position as an anatomic matter of controversy, the histological aids, still rather imperfect, left ample room for personal interpretation attempts to take place. We may in the main distinguish three different efforts in explaining the circulatory conditions in the red splenic pulp: the theory of the open circulation, the theory of the closed one and the theory of the divided circulation.[1]

The Open Circulation

In 1860, Billroth writes in his publication on the human spleen in connection with his description of the capillary splenic veins, i. e. the spleen sinuses: "Vermuthlich endigen die arteriellen Capillaren in dem intervasculären Gewebe mit offener Mündung und die Blutkörperchen werden durch letzteres vielleicht hindurch und durch die möglicherweise durchgängigen Wandungen der capillären Venen in das Lumen der letzteren hineingepresst." In this sentence Billroth has advanced the hypothesis about the open — even called the intermediary — circulation in the red pulp.

In 1862, Stieda found that the injected matter, emptied from the arteries, lay in an intercellular network within the pulp cords. He asserted, however, that this network did not consist of capillaries in a usual sense, but that the blood — as the glue in the injection experiments — passed freely to and fro between the cells of the pulp cords. He compared its distribution with that of the bile capillaries between the liver cells. Stieda found, also, that it was impossible to fill the artery when injecting from the vein. Pointedly he vindicated that there is an open circulation in the spleen.

In a monograph on the spleen of 1865 Wilhelm Müller describes the minute structure of this organ. He examined several human spleens and some fifty derived from different animal species, from fishes to apes. This he did after vessel injections as well as with ordinary histologic methods. He pointed out the constantly rich presence of erythrocytes everywhere in the red pulp, and he calls

[1] This term will in the following be used to designate the special type of circulation, which, according to the opinion of the author, characterizes the function of the spleen sinus.

this fulness of blood a natural injection. Even when using artificial injection matters he found that they filled up the intercellular spaces of the pulp cords, which he calls the intermediary pathways of the blood. This MÜLLER could establish with respect to all examined species. On the other hand, he could not confirm BILLROTH's discoveries of the spleen sinuses, but held that the blood from the intermediary pathways entered the finer veins, which began, as he found, with a perforated wall.

MÜLLER's view was at this time shared by most histologists. So HENLE, for instance, who in his *Handbok of Anatomy* (1873) arrives at the conclusion that the open circulation is the most probable one. FREY (1874) holds the same view. After injection experiments BANN-WARTH (1891) and HOYER (1894) came to identical results. In turn, LAGUESSE (1891) suggests the explanation for this peculiar type of circulation, viz. that the development of the spleen has ceased at an embryonic stage.

In 1901, WEIDENREICH introduced a new injection method. Into three rabbits and one dog he injected avian erythrocytes intravenously, and then killed the animals after different lengths of time. Immediately after the injection he found nucleated red cells in the pulp partitions but, on a later stage, a greater number in the spleen sinuses. This result is rather difficult to explain. However, he concludes that the blood is first emptied into the pulp cords and later forced into the spleen sinuses by means of diapedesis. But he also observed direct communications between arterial capillaries and spleen sinuses in a few cases. He, therefore, arrived at the conclusion that in one and the same spleen there may exist partly an open circulation, this being the rule, and partly direct communications between capillaries and spleen sinuses.

MALL (1903), who previously had held the theory of closed vessel-pathway, now adopted WEIDENREICH's view upon having continued his injection experiments. Having paralyzed the trabecular muscles through injection of sodium nitrite, he found masses of erythrocytes extravascularly, in the contracted spleen, on the other hand, only few red cells outside the spleen sinuses. Moreover, he pointed out direct connections between arterial capillaries and spleen sinuses. These connections he apprehended as a series of pulp spaces, which possibly could be called undifferentiated capillaries, comparable with those that occur in the fetus. He believed the spleen sinuses to have

a porous wall. During injection from the vein, he found that certain coloring matters passed through the sinus wall with ease, others with difficulty. Cinnabar, for one, passed smoothly into the pulp cords, ultramarine blue hardly and chromic yellow not at all. MALL was a pupil of THOMA, who is to be quoted later.

In 1922, NEUBERTH examined spleens of swine, dogs and cats. After having excised them he perfused them, first with Ringer solution of body-temperature and then with fixation aids. By this he brought about an artificial spleen congestion. WORONIN has indicated the method in 1898. NEUBERTH found, moreover, that the cellular content of the pulp cords get washed away during the perfusion experiment. This he thought to indicate that there does not exist any closed vascular pathways. The same was the results of his microscopical examination, where he could not find any direct connections between arterial capillaries and the spleen sinuses. He found, instead, that the arterial capillaries were terminating in funnel-shaped formations, the wall of which was gradually broken up and then passed over into the texture of the pulp cords. The spleen sinuses began in like manner as funneled distensions of certain pulp spaces. Between these funnels there was an open circulation. NEUBERTH admits, however, that the arterial funnels have a certain resemblance to the venous ones and for that reason it is not improbable that he, as a matter of fact, is speaking about the both ends of a spleen sinus.

BARCROFTS rediscovery in 1923—24 of the splenic reservoir function increased the interest in the splenic circulatory problem. Guided by the localization of injected Indian Ink, MILLS in 1926 arrived at the definite conclusion that the circulation must be open, for the Indian Ink particles became congregated everywhere in the pulp cords. If first bringing about a dilatation of the spleen and, afterwards, performing a perfusion of it from the vein with a pressure at 80 mm Hg., he could produce a flow from the arterial cannula. This flow decreased upon adding epinephrin but increased upon potassium nitrite. That such a backward perfusion washed away the cellular content of the pulp cords, this MILLS believed to be an evidence for the theory of the open circulation, just as NEUBERTH thought, having perfused in the opposite direction.

In 1927 MCNEAL, OTANI and PATERSON repeated the experiments of WEIDENREICH with injection of avian corpuscles. Besides, they examined a great number of human spleens fixed by WORONINS

method. They found very few nucleated red cells in the spleen sinuses but came across some of them in a diapedetic position in the sinusoidal wall. They, therefore, considered them to be on their way from the pulp cords into the sinuses. These findings they interpreted in favor of the open circulation, as well as the fact that they never saw any direct communication between sheathed capillaries and sinuses.

Making use of WORONINS method, ROBINSON studied in 1930 the circulation in cat spleens, and came to the same result as NEUBERTH, viz. that the rise of the veins, i. e. the spleen sinuses, was formed by funnel-shaped conglomerations of wide pulp meshes without every trace of connection with the arterial capillary. These funnels were longer in man than in dogs and cats. There they were also provided with myriads of small slits.

In sections stained with iron hematoxylin TEITEL-BERNARD, in 1930, thought to find pictures showing clearly how erythrocytes from the sheathed capillaries left the vessel pathway and passed through the pulp cords in order to invade the spleen sinuses.

MCNEE (1931) injected yeast-cells, grown to the same size as red corpuscles, in a dog. Generally, above all in dilated spleens, he refound the yeast-cells in the pulp cords, but after splenic contraction which he brought about electrically, they appeared in the sinuses. The reason for this later finding MCNEE regarded to be the fact that the spleen does by its contraction push out the pulp content into the sinuses. He measured the venous splenic pressure during contraction and found it amount to 190 mm Hg. However, he also found that many arterial capillaries issued directly into the sinuses, and so he believes that there may be both an open and a closed pathway in the same spleen. This view was already held by HUECK in 1928. He created the slogan "Flutkammern" (storing tanks) for the follower of the open theory, meaning the pulp meshes, where the reservoir-blood is shut off from circulation. At the cost of the vessels the capacity of these tanks might be augmented; their volume was thought to be correlated with the state of dilatation of the spleen. In the contracted spleen the pulp meshes are compressed and the blood passes the shortest way from the arterial capillary into the spleen sinus through the pulp cord. Contrariwise, in the dilated spleen the tanks get enlarged and capable to receive most of the incoming blood.

HUECK states that there are two states, in which the capacity of

the spleen is increased. In the arterial hyperemia, the first of them, much blood is flowing in because many capillaries open in the pulp spaces. The sinuses get compressed from outside and cannot receive a sufficient quantity of blood. In the second state, venous hyperemia, there is a stoppage to the outflow of the blood. The volume of the sinus then gets insufficient and the blood accumulates in the storing tanks.

McNee also studied the pathway of the blood in living spleens. In an experimental animal he transilluminated the thin splenic edge and observed its appearance through a dissection microscope. He saw erythrocytes lying stationary at certain places but at others passing rapidly through the red pulp. Any more penetrating analysis by this method McNee has, as it seems, not achieved. His contribution was the findings of the immobile erythrocytes.

In 1935, von Herrath carried out a comprehensive investigation about the minute anatomy of the spleen with a quantitative estimation of different elements in various animals. Some spleens had been perfused before fixing. Only in exceptional cases, he was able to find evidence for direct communication between arterial capillaries and spleen sinuses. Guided by the cellular content of the sinuses, he distinguished three different phases of sinus activity — flowing sinuses, working sinuses and storing sinuses. In the first phase, he found no cells at all in the sinuses; in the second, there was blood of a peculiar composition, different from that of ordinary circulation, and numerous white cells traversing the sinusoidal wall. The sinuses belonging to this particular phase he considered to be shut off from the circulation. Finally, those sinuses, which were crowded with erythrocytes he believed to be in the storing phase. This differentiation of the spleen sinus activity into various phases with changing histologic appearances, signifies a new conception of the circulatory problem.

The mechanism that determines the distribution between flowing and working sinuses, von Herrath refers to the arteries. Agreeing with Henschen and Reissinger (1928), he certainly thinks that the muscular splenic arteries are capable of shutting off greater or smaller parts of the spleen from circulation. As evidence there is given the occurrence of spleen infarction without the finding of any embolus. Tho dependent on the size of the artery in which the stoppage is located, it is possible that greater or smaller parts of it may be shut off from the supply of blood. von Herrath holds that the red cor-

puscles reach the sinuses through the pulp partitions and that this takes places in performing an open circulation. He believes also that a local contraction of the reticulum between the capillary and the sinus may constitute a stop-mechanism.

Summing up, one can say that investigations carried out by a great number of scientists right down to our own days suggest that the pathway of the blood in the spleen should be different from that of other organs. The blood should there leave the endothelium-lined vessels, as a rule the finest arterial ramifications, and saturate the red pulp. This unique pattern of circulation the followers of the open circulation consider to constitute a principle well adopted to its purpose. They believe that by an intimate contact between blood and pulp cells — tho yet greatly unknown with respect to their functional nature —, which is thus established, the spleen may carry out its physiological activity more effectively than if the blood passed these cells in a closed system without achieving that contact. The peculiar conditions about this circulation they regard as an unexpressed proof of the fact that the splenic function must be to act upon the blood.

The Closed Circulation

In opposition to the majority of investigators adhering to the doctrine of open circulation there appeared some histologists who held that the blood in the spleen, as in other organs, traveled along in a closed pathway. As a matter of fact, Axel Key vindicated this view already in 1861. By means of injections from both artery and vein on calves, swine and sheep and even human spleens, he found that the *penicillii* poured its blood into a widely ramified capillary network within the red pulp. This constitutes, in his opinion, the principal part of the red pulp. In the meshes of the network he found spaces which he considered belonging to the lymphatic system but he failed in pointing out their connections with the lymphatics. Those very spaces consisted actually of spleen sinuses as far as one can now ascertain from his drawings. Here Key has made the same mistake, as Gray did earlier, by interpreting the injected intercellular system between the pulp cells as a capillary web in a usual sense. That the intercapillary pulp meshes, i. e. spleen sinuses, could never be injected, this he took as evidence showing that they could not belong

to the circulatory bed; on the contrary, he thought they make up a part of the lymphatic system. One exception was a few human spleens, where KEY, however, interpreted the injected spleen sinuses as the result of an artifact extravasation. He thus took his stand as an opponent to BILLROTH'S hypothesis about open circulation. Nevertheless, he is through his experimental results a supporter of this view even tho he argues against it.

After BILLROTH in his publication of 1860 had introduced the theory of the open circulation, he continued, however, his spleen studies and arrives in a new work (1862) to an altogether different understanding about the true nature of the vessel pathway. Upon injection experiments, especially those carried out on child spleens, he came to the very definite conviction that the arterial capillaries everywhere opened into spleen sinuses. The common finding of red corpuscles outside the sinuses he considered due to the fact that the sinusoidal walls at high venous pressure turned permeable for erythrocytes as also for the least particles of the injection matters.

In the very same year BILLROTH received one supporter of his new hypothesis. It was SCHWEIGGER-SEIDEL, the discoverer of the sheathed capillaries. On the basis of his own experiences he takes a rather critical stand with respect to injection experiments. He doubts very much the existence of an intercellular capillary network which KEY and STIEDA have described. At a meeting with the latter he had the occasion of studying his injection methods close at hand. Afterwards, he made several experiments, using his technics, and received the same results. Yet he regarded this injected intercellular network as an artifact, brought about through an extravasation of the injected matter. Preparations worked out with the greatest care showed him that in man there must absolutely be a direct communication between arterial capillaries and spleen sinuses. SCHWEIGGER-SEIDEL is also the first one to acknowledge BILLROTH'S discovery of spleen sinuses, tho criticizing his measures; for he has himself found the average width to be ca. 35 micra. He gives, too, an adequate description of annular fibrils and spleen sinus endothelium.

Toward the end of the 19th century several separate investigators adopted the theory of closed splenic circulation. In 1867, KÖLLIKER succeeded, like BILLROTH earlier, in showing that there in the child spleen is a direct connection between arterial capillaries and sinuses. This he demonstrated having injected the artery; he also points out

the importance of low injection pressure. But, in spite of it, he often got extravasations at the end of the arterial capillary. Using the injection method KYBER (1870) and KRAH (1877) were, likewise successful in pointing out an immediate communication. In turn, G. RETZIUS (1886) maintained that by careful injection it is quite possible to avoid extravasations. In 1889 VON EBNER believed himself finding that there was a very delicate membrane extended between the annular fibrils. Its existence made him an adherent to the doctrine of closed circulation. In 1900, VON SCHUMACHER grants that he is right, and says it has been especially easy for him to show the same in the woodchuck.

During the last years of the 19th century THOMA and his school at Dorpat were busy with injection experiments on spleens and performed a penetrating analysis about the problem of pulp circulation. Having brought about an artificial congestion by tying the splenic vein, his pupil SOKOLOFF in 1888 studied the red pulp in rabbits, dogs and cats. After an extension of the engorgement during 15 minutes, he found a dilatation of the spleen. Histologically, this took the form of a distension of the spleen sinuses, which got crowded with erythrocytes, while the pulp cords had scarcely none. They showed, however, an increased breadth as well as a distension of the intercellular spaces. This he interpreted as being dependent on an edema, which had arisen by filtration of plasma through the sinusoidal walls as a natural consequence of the congestion. Only when the stasis had lasted more than 15 minutes, the erythrocytes began to escape through the sinus wall.

SOKOLOFF believes that his findings argue against the theory of open circulation. Should this be an established fact, then one ought to have met with a congestion of red cells *in the pulp cords* and not in the spleen sinuses. He considers the sinus wall to be a permeable membrane. Its permeability he finds variable in different animals. It is largest in cats, next dogs and then rabbits. In his opinion, the wall consists of a layer of longitudinal cells surrounded by connective tissue. Even a slight degree of engorgement should be enough to separate the endothelial cells from one another so that slits would be formed in the wall. He could not decide whether the slits are normally found or should be considered pathological phenomena. He is, most of all, inclined to believe they appear physiologically and so let injection matters during experiments pass into the pulp cords.

Sokoloff held, moreover, that it was quite possible for plasma to flow from arterial capillaries by way of pulp partitions into spleen sinuses; but he does not seem to have reckoned with any transition of blood fluid through the sinus wall by the pulp cords into the veins. That corpuscles do not leak out into the pulp partitions, while experiments are going on, he considers due to the fact that the pressure inside this tissue rises as a consequence of plasma filtration and that this raised pressure constitutes an obstacle for continuous extravasation. The pulp edema is, according to his view, resorbed by the lymphatics. The slits in the sinus wall may, consequently, be regarded as a communication between the blood and lymphatic pathways. The profusion of red corpuscles in the pulp cords, as seen in histological sections, he looked upon as being a stasis phenomenon arisen *in agone*.

His findings were reexamined by three investigators belonging to the same school — von Kalenkiewicz (1892), Wicklein (1888) and Panski (1890). On the whole, they were able to confirm his findings. They saw how the pulp edema appeared, first of all, in the perinodular areas, and considered it due to the fact that at this place a great number of lymphocytes passed into the circulation. Wicklein showed that if the congestion ceased after an excessive crowding of the pulp cords with red cells, then these suddenly disappeared without being hemolyzed.

Shortly before death, Thoma summarizes his experiences and investigations about spleen circulation. The work got published posthumously in 1924. By using soluble dyestuffs in their injection experiments, he concludes, some histologists have refound them in the pulp cords and so regarded the theory of the open circulation as proved. But, on the contrary, by using dyestuffs of corpuscular nature, others have run across them intravascularly; and to their minds the closed character of the pathway stands out very clear. However, the finding of injected matters outside spleen sinuses, indicates only, as Thoma holds, that the walls of the splenic vessels are permeable. It does not tell us through which of these vessels the extravasation occurs. In other words, the withdrawal may just as readily take place from arterial capillaries as from spleen sinuses. That the artery cannot be injected from the vein Thoma considers due to the great permeability of the sinus wall. As another source of error he regards the assumption that aggregations of corpuscular

elements lose some of their fluid matter which passes on into the pulp partitions. These elements, then, form a hindrance in the vessel, which will, of course, rupture during continued injection. In such a way, extravasation develops and a false picture will arise. THOMA has, besides, found the vessels in child spleens to be more permeable than those in dogs and these more than those in rabbits.

He holds the opinion that while passing through the red pulp, plasma and erythrocytes may get separated from each other. Already SOKOLOFF had made such an assumption for the purpose of explaining the rise of pulp edema when tying the splenic vein. His hypothesis that plasma during the congestion filters through the sinus wall, is quoted by THOMA, who, however, believes that such a separation may even take place during the travel of the blood through the arterial capillary. Plasma should, then, pass through pulp partitions into the sinuses while the erythrocytes realized an intravascular passage. According to THOMA's theory, the flow of plasma from the pulp cords into sinuses should counteract the tendency of the red cells to force their way out through the sinus wall, a tendency which has its origin in the porous structure of the sinus wall. His conclusions concerning the filtration through the sinus wall do not seem to be based upon experimental investigations.

Another adherent to the doctrine of closed circulation was HELLY (1902 –03). He got involved in a heated debate with WEIDENREICH. Having reexamined HELLY'S preparations, WEIDENREICH found evidence for his own view, viz. that the pathway of the blood is, chiefly open. When HELLY, on the other hand, got the opportunity to study WEIDENREICH'S sections, he found in them support for the opposite view, which to him appeared as the most probable. He repeated the experiments of WEIDENREICH with injection of avian corpuscles and found them, in most sections, to be located within the spleen sinuses, consequently, just the reverse to what WEIDENREICH found. Occasionally, HELLY could see how they forced their way out through the sinus wall; however, it is not given any account of the method how to decide their travel-direction. While experimenting with larger nucleated erythrocytes derived from the frog, he refound them intravascularly, almost without any exception. Hence he concluded that the pathway is everywhere closed and that the blood everywhere passes between endothelium-lined walls. At the same time he admitted, nevertheless, that the spleen vessels present a high degree

of permeability. The extravasation of red corpuscles there assumes, he states, such an enormous degree that one may really speak of physiological echymoses.

Nisimaru and Steggerda, disciples of Barcroft, challenged in 1932 the validity of Stieda's thesis that one cannot inject the splenic arteries from the vein. By means of an specially construed pump, they could send defibrinated blood the very way they for the moment wanted, and could even perfuse the organ with fixation matters. The experiments were performed on dogs under narcosis. It appeared that retrograde perfusion could only be brought about with a pressure that amounted to 40—50 mm Hg before any flow through the arterial cannula was established. They point out that the physiological aspect of this pressure does not need to be emphasized. For McNee could record from three to four times higher pressure than that in the splenic vein during contraction of the spleen. When adding Indian Ink to the perfusing blood, they never found it in the Malpigian corpuscles, the capillaries of which seemed, therefore, to offer greater resistance to the flow of perfusion. After every backward perfusion they changed its course into its normal direction. The state of circulation were, then, found intact. The outflow of arterial cannula increased in volume at rising pressure. Only when it passed the limit of 40—50 mm Hg, the spleen began to dilate. "The remarkable fact was that when the pressure rose, the volume of the spleen due to accumulation of blood first increased but little, but at a rather higher pressure increased considerably for a given increment of pressure." — "It may be that the venous sinuses are at first distended, and after the pressure in them has reached a certain height, it becomes equal to, or slightly higher than the resisting pressure of the smaller arteries and allows the blood to escape." From this it follows that they adopt the theory of closed circulation.

All investigators that hold this view of the closed pathway have, however, admitted how easily one meets with extravasations from the splenic vessels, above all distally from the sheathed capillaries. But these extravasations they consider an artifact phenomenon while their opponents, adhering to the doctrine of open circulation, regard them as representative for the pathway of the blood. The discussion about injection experiments turned out being, to a great extent, a wordy warfare, strongly colored by attempts at subjective interpretation. From this controversy, at times passionate, one thing,

however, became clear, viz. how difficult it is to examine the spleen structure by means of prevailing histologic methods.

The Divided Circulation

As indicated above, there is a third possibility to reckon with in explaining the pathways of pulp circulation. SOKOLOFF, THOMA and MALL presumed that erythrocytes and plasma, got separated while passing through the pulp so that plasma, or at least a part of it, passed from the sheathed capillary through the pulp cords into the sinuses while red corpuscles followed an altogether closed pathway. This assumption seems to have risen from attempts to make clear those extravasations constantly recurring during injection experiments. SOKOLOFF, however, outlines another more well-grounded separation mechanism when he accepts a filtration of plasma out from the sinuses in order to explain pulp edema arising during engorgement.

The most important investigation made about splenic circulation, since BILLROTH's discovery of spleen sinuses in 1860, has, according to my opinion, been accomplished by KNISELY, an American anatomist, in 1936. He performed some very valuable observations concerning the splenic vessel pathways and gave an account of the results obtained by an altogether new method for the study of circulatory problems. By directing a powerful beam of light beneath the free-dissected spleen in an experimental animal under narcosis by the aid of a bent quartz rod he secured the possibility of studying the vital circulation, through microscope, under so physiologic conditions as obtainable. He made his observations by using a water immersion objective with a magnification up to 600 ×. Making use of this method, called transillumination, KNISELY examined spleen-circulation in cats, rats and mice. His findings derived from these animals were, in principle, of the same nature in all of them.

Emphatically, KNISELY points out that the spleen sinuses constitute the functional unit of the spleen. These peculiar formations are morphologically well defined, and must not be confused with capillaries, veins or arterio-venous anastomoses. They perforate the pulp in all directions, like tunnels of changing wideness, drilled in cheese. Between them one will find the pulp cords, the breadth of which seems to be correlated to the diameter of surrounding sinuses. During transillumination it is possible to distinguish a bright

refractive vessel-wall everywhere within the splenic pathway, i. e. circulation is everywhere closed. The connections of the *penicillii* with the veins consist of two kinds of vessels: partly the capillaries of usual histologic appearance, which issue directly into the collecting vein, and partly the spleen sinuses, which for this organ constitute quite unique and fundamentally characteristic formations. They appear now separate, now coupled to series of sinus systems, where, however, the individual sinus is maintained as a functional unit.

Knisely's account of the spleen sinus activity is the most exciting part of his investigation work. In order to understand how this is possible, one must first of all describe some powerful physiologic sphincters, which he found located in the vascular arborization of the spleen. They appear at different hights of the arterial distribution; the ellipsoids form, for instance, vigorous ones. At the openings of the sheathed capillaries into sinuses, as at the sinus outlet into the vein, there are similarly found energetic sphincters, which, however, cannot be morphologically recognized. Knisely found that the flow of blood was directed by rhythmic contractions of these sphincters. So it was, for instance, within a *penicillus* sector having three ramifications, one of them constantly open while the other two were closed. Every ramification was held open about 50 minutes.

Circulation through spleen sinuses shows two different principal types. During one of them blood passes directly through the sinus into the vein, whereas, during the other, which shows cyclical variations of the width of the sinuses, a storing of red corpuscles takes place. The cyclical phase is divided into four sections. It starts with a short filling phase, initiated through contraction of the efferent sphincter at the sinus outlet. The sinus is, then, rapidly loaded with whole blood from the sheathed capillary and, consequently, dilates. At the same time there starts a filtration of plasma out through the sinus wall. In consequence of that the red cells get inspissated. When the sinus is almost filled, the afferent sphincter closes and the next period, the storing phase, begins. During this, which may last up to 10 hours, the sinus is altogether shut off from circulation. The sinus content now consists of a thick mass of erythrocytes. While still in filling phase the surrounding collecting veins contain only few red corpuscles but plenty of blood fluid. The exact point where

plasma re-enters the circulation KNISELY failed to discern. At the outset of the third phase the efferent sphincter first opens very quickly, then the sinus content wells out as a thick porridge into the vein, where perhaps clear plasma comes from another area at which sinuses pass into storing phase at that very moment. Then the afferent sphincter opens also and whole blood rushes as a pulsating stream into the sinus washing out the remains of the erythrocyte mass. As its width decreases the sinus returns to passing phase.

Any slits in the sinus wall KNISELY was not able to ascertain. During transillumination separate red corpuscles escaped through the sinus wall, which, by the fact that they got deformed, seemed to resist their passage through it. They forced their way out always one by one and never several abreast. So KNISELY believes that if slits exist, they must be very small. The plasma transfer through the wall must, on the other hand, take place without resistance. He holds it probable that the spleen sinus wall is capable of great, yet unknown, variations of its permeability. Having squeezed their way through the wall into the pulp cords, the erythrocytes trickle slowly forward, one by one, but never congregated in a stream. From their moving manner which is rather squirting, KNISELY concludes that they travel through narrow channels.

These investigations show that it must be wrong to speak about open or closed circulation, apart from the stream in the straight capillary. As a matter of fact, circulation goes on after an altogether new discovered principle, which at best has before only been suggested (cf. SOKOLOFF and THOMA), viz. red corpuscles and plasma travel separated from one another. His investigational results have, despite its revolutionary content, not aroused the interest one might have expected. Findings and speculations of earlier spleen histologists could, nevertheless, scarcely maintain a stand before the scrutiny of transillumination. His discoveries gave at the same time one functionally acceptable explanation of the rather elaborate vascular structure. After his publications, there ensued a standstill in the discussion about the splenic circulation, and one has been waiting for some more reports from the author of transillumination. His main results should, according to a brief communication, have been confirmed by PECK, who used the same method in an afterexamination. This work which in 1944 was announced "in press", has, however, to the best of my knowledge, not yet appeared.

In 1941 McKenzie, A. O. Whipple and Wintersteiner made the only after-examination of Knisely's results which as yet has been published. They used the transillumination apparatus as construed by Knisely, but were, in spite of it, unable to confirm his findings. They found the red pulp positively red on account of the rich occurrence of erythrocytes in it. From the capillaries that ended in clublike *ampullae* the blood emptied, according to their opinion, into the meshes of the pulp partitions. These spaces formed channel systems constantly changing with respect to their width and running course. Within those channels they found the velocity of the circulation greatly variable, and judge that random and irregular storing of erythrocytes may take place in certain pulp spaces depending on the fact that they found aggregations of white corpuscles shut off parts of the pulp pathways from the streaming blood. When "the white stopper" gave way, a stream of red cells was seen entering the sinuses through the wall from outside.

McKenzie and his collaborators deny the occurrence of any rhythmic sinus activity, so exactly described by Knisely. In addition to what is told about the casual arrest of circulation through aggregations of leucocytes, they found no rhythmic activity taking place in the splenic pulp. On the other hand, there is a certain, macroscopically observable, shrinking of the spleen as a whole, known as rhythmic contraction of the spleen and studied by the Barcroftian school. During transillumination they considered this rhythmic contraction correspond to that pressing the erythrocytes out from the pulp cords into the sinuses. Just as the animal was dead they found, likewise, the contracting spleen pouring the blood masses of the pulp partitions into the sinuses. In their view they mean to have support from Barcroft, who found the dead spleen in dogs being but one third of the living spleen.

The conclusion of McKenzie and coworkers is manifesting their unableness to verify Knisely's observations. They declare themselves convinced about the value of transillumination as a workable method for histologic investigations, but point out, at the same time, the immaturity still hanging on to it. This consists of optic difficulties, especially that of differentiating various structures, which constitute the soft mass in the living spleen where it is impossible to draw any distinct lines of demarcation. This does, in their opinion, lend the subjective factor greater freedom for personal interpretation.

It is regrettable that the clear and concise picture of the splenic

circulation, KNISELY has given us and that this very picture which makes the results obtained by FÅHRÆUS and BERGENHEM so intelligible, has not been substantiated and confirmed by these after-investigators. But there are considerable herculean tasks along the way to the mastery of transillumination technics. This I have had the opportunity of observing close at hand when MELLGREN, in 1941, here at the Institute sought, but in vain, to eliminate the source of errors permanently emerging while using that method and so, because of these technical difficulties, had to abandon his intention to re-examine KNISELY'S results. The suspicion is, consequently, near at hand that methodological errors have exercised some influence upon the results of MCKENZIE and his collaborators, which bewilder and tear into pieces KNISELY'S distinct picture of splenic circulation.

One more investigation is here to be mentioned on account of its relations to the results of KNISELY and MCKENZIE. It is SNOOK, who, in 1944, by making use of prevailing histologic methods carried out an examination on the reticular framework in Guinea pig's spleen. Using a silver staining method, as modified by himself, he made a waxy reconstruction of the spleen pulp. He found, however, no signs indicating an open circulation; the arterial capillaries mostly opened into spleen sinuses. As the reason for KNISELY'S and MCKENZIES conflicting results he partly regards the fact that while MCKENZIE used mice as experimental animals, KNISELY was, in the main, working on rats. While the former have a poorly developed sinus system, the latter have an abundant one.

Few structural questions have been debated with such enthusiasm and eagerness as the problem about splenic circulation. But one has, just the same, not yet attained to any final solution, and so the question reappears at regularly intervals. Even during elaborate performance the injection experiments implied with certainty some unphysiologic injury to the fine structural details. The histologic examination is doomed to failure on the basis of the involved vascular architectonic. Nor has transillumination, tho published ten years ago, up to this time afforded any results capable of but one interpretation.

The Minute Structure of Spleen Sinuses

On the preceding pages there has been stated how some investigators have arrived at the conclusion: either that whole blood passes the spleen sinus wall or that plasma filters out through it (Sokoloff, Knisely). In order to understand how this may be possible, it is necessary to give an account of the minute structure of the sinus wall. Many investigators have, besides, not only spent their time in studying circulation but have, likewise, manifested much interest for the anatomic details. Their works are partly of a purely descriptive nature without aiming at the functional conditions.

The main traits of the minute anatomy of the sinus wall were already known to Billroth. He describes and reproduces two layers forming the sinus wall. One of them, the outer, consists of extremely delicate annular fibrils running in circles, and the other, the inner endothelial layer, is composed of spindleshaped cells. It is of interest to notice how the regular interval between the annular fibrils seems to have impressed already Billroth since he measured these distances finding the value 10 micra. He also calls attention to the fact that the sinus wall in new-borns is different from that of grown-ups. Here the difference between annular fibrils and reticular structure of pulp meshes is exceedingly small, and the whole pulp takes, thus, on the appearance of an uniformly developed network so that the existence of spleen sinuses is mostly altogether veiled. A similar arrangement Billroth also found in spleens from sheep and oxen, in which he had much difficulty in finding open sinuses. This observation of his, have in the literature, as it seems, in later years received no attention.

Also Schweigger-Seidel gives a similar account on the structure of the sinus wall but does not mention the child spleen. The circular fibres have, since the time of Henle (1873), been called annular fibrils because of their ringformed course around the sinuses.

In 1901, Weidenreich thoroughly describes the annular fibrils and endothelial cells. The latter he names rod-shaped cells. He finds their length runs up to ca. 100 micra and their nucleus, beeing broader than the cellular body, extends into the sinus lumen. It is, according to him, not covered by any protoplasmic layer, but being, instead, streamed around directly by the blood. In sections or isolated splenic

material WEIDENREICH found the nucleus presenting two longitudinal streaks, which he interpreted as folds in the nuclear membrane. The protoplasm of the cellular body he found to be, on the contrary, without any structure. He considered these cells having contractile properties because he was of the opinion that the trabecular and capsular muscles in man were too poorly developed to be able to empty the spleen by their contraction.

According to WEIDENREICH, the intervals between the rod-cells increase during sinus dilatation. They may vary from 2 to 3.5 micra. Like BILLROTH, he showed interest for the interstices between the annular fibrils which he, however, found rising not higher than to 4—5 micra. Often he observed diapedesis through the slits between rod-cells and annular fibrils. He believed the slits arose through such diapedesis. In splenic engorgement or increment of the blood volume spleen sinuses distended to the utmost that these cells could not contract and that, likewise, a hemorraghic infarction gradually developed.

In 1909, there were two investigations carried out about the minute anatomy of the sinus wall, one by the great French microscopist JOLLY and the other by a Russian lady, ANNA MANGUBI-KUDRJAVTZEVA. On the whole, they arrived at the same results, viz. that the sinus wall is built up a by circular layer of annular fibrils and, inside this, by a longitudinal stratum of rod-cells. At cross-sections from sinus one may, according to these investigators, see long offshoots of cells fasten on the inside of the annular fibrils as the cogs in a wheel, tho with them turned against the axle. In cross-sected sinuses, having the same diameter, they saw often various numbers of square-cut endothelial cells. In general, they are, however, more numerous in wider sinuses. In formulating this assertion they may, however, possibly have confused spleen sinuses and intralobular veins. Like WEIDENREICH they find the nuclei project into the lumen. These are not localized at the same level but their projections form a spiral border at the sinus wall. On the outside of the rod-cells, one distinguishes crescent-shaped incisions at regular intervals corresponding to the attachments of the annular fibrils.

JOLLY and MANGUBI-KUDRJAVTZEWA disagree, as a matter of fact, only about the existence of a thin basal membrane extended between the fibrils. At this membrane the rod-cells are situated. The occurrence of such a one, earlier found by VON EBNER and SCHUMACHER,

is vindicated by MANGUBI-KUDRJAVTZEVA but emphatically denied by JOLLY. Tho experimenting with all conceivable staining methods yet he never succeeded in observing any such a membrane. As evidence for its existence, however, MANGUBI-KUDRJAVTZEVA cites the fact that by using iron hematoxylin stain, she has managed to show basal plates in the rod-cells. They appeared only between the attachments of the annular fibrils, i. e. between the incisions. Due to their occurrence the cells, as seen from above, in longitudinal sections become a striated appearance. She thought these basal plates being a part of her basal membrane. The slits between the rod-cells she considered produced by diapedesis of leucocytes through the basal membrane. This striated structure in the endothelium has, subsequently, been mentioned by a few authors — FOOT (1927) and HARTMANN (1928).

In 1911 MOLLIER published the results of a comparative histologic study about the wall of the splenic sinuses in man, ape, dog, cat, rabbit, sheep, cattle and swine. Before fixing he washed through the spleen according to WORONIN. Only in man, ape, dog and rabbit MOLLIER found the elements in the sinus wall to be regularly arranged in the fashion of a grid. He holds that separate endothelial cells do not exist, but that, instead, the inner layer is formed by a surface syncytium, which not in principle differs from the cellular syncytium of the pulp cords. Between the most prominent longitudinal elements there are namely cross-connections at the level of annular fibrils. The lumen he regards as a series of pulp spaces having the same wideness.

Man and ape have no longitudinal reticular fibrils in their sinus grid, only a few anastomoses running in an oblique manner between the annular fibrils. In all other examined species MOLLIER found, however, longitudinal fibrils, in dog and rabbit running regularly, in others altogether scattered. MOLLIER points out the congruence prevailing everywhere between the reticular fibrillar network and the endothelial one. This he considers point to the fact that we have to deal, indeed, with a reticular syncytium.

MOLLIER believes, moreover, that a dilatation of the spleen sinus with an enlargement of the holes (= stomata) in the wall furthers the diapedesis of cells. He reproduces also striated endothelial elements, yet without mentioning the staining used (MALLORY?), and regards the brighter parts of the striation, localized straight before

the annular fibrils, as remains of cross-connections between the longitudinal elements of the syncytium. MOLLIER rejects the thought that perfusion before fixing could have brought about artifacts; for a comparison between spleens washed through with others not, reveals in both cases the same picture, "nichts ist beschrieben, was nicht auch an solchen Präparaten zu sehen ist".

The perforated nature of the sinus wall, imagined by earlier authors, may through MOLLIER's classical work be considered as fully proved. In DOWNEY's *Handbook of Hematology*, KLEMPERER in 1938 suggests following significance for circulation of the holes in the sinus wall: "However, the width of the stomata is variable and is influenced by various factors. For instance, contraction of the spleen will cause a diminution of the size of the pores, whereas increased tension within the vascular channels provoked by elevation of the arterial or venous pressure will result in a dilatation of the stomata within the vascular wall. Furthermore, changes in the colloidal phase of the cytoplasmic reticulum with concomitant swelling or shrinkage of the trabeculae of the grid must necessarily cause either a decrease or an increase in the width of the stomata. It is evident that if the pores of the vascular channels are wide open, the blood will escape freely into the surrounding pulp spaces, whereas if they are narrow, the blood will remain within a pathway separated from the surrounding spongy spaces of the pulp by a perforated but very narrow meshed wall." In this connection it may be mentioned that HUECK in 1928 found the intervals between the rod-cells not exceeding 2—3 micra; in contracted ones only about 1 micron but still distinct. How he got these figures, is not told. His disciple NIEDRMAYR found, in 1928, the same intervals greater in spleens washed through than in those not; they rose to 6 micra in the perfused spleen.

Using sections impregnated with silver, FOOT examined in 1927 the structure of splenic reticular framework. In some cases he perfused the spleens before fixing. He found the reticulum of the ellipsoids being very fine and felty. It was closely connected with the pulp cord reticulum. The larger veins ran in reticular sheaths, the intralobular ones were surrounded by annular fibrils, which were approximately parallel. No longitudinal reticular fibrils do ever come in contact with the vessel wall. The stomata in the sinus wall were wider than those in the venous wall. The affinity to silver of the annular fibrils he found more varying than that of the other

pulp reticulum. It got reduced through perfusion with physiologic saline.

Foot regards the pulp reticulum as possibly having less elasticity than that of the vessels because after spleen engorgement it is ruptured in fine pellets. With respect to endothelium he, also, considers it contractile. As evidence he cites his finding of a slight striation as well in sections as in isolated material. This is based on darker segments between the annular fibrils; as seen in tangential sections they resemble *H.* It may be that they consist of densifications in the cellular membrane, as they seem to be situated on the peripheral surface of the cell. The rod-cells always pass longitudinally except at the departure of lateral ramifications, where their running course turns more radiating and complicated. At the outlet of the sinus into the vein they get more squeezed together. In maximally wide sinuses the endothelium cells no longer run parallel but in all directions.

In 1935, von Herrath described different structures in various animal spleens. Like Weidenreich, he regards the trabecular function in man as that of a pure framework; their contractile elements are insufficient to produce a spleen contraction. The contraction must, instead, be brought about through sinus and reticulum. Man has, like rabbit, rather weak trabecular muscles, but, according to von Herrath, a well developed sinus system. In dog, cat, sheep and swine this is, however, much less evolved but here the volume of the pulp partitions predominates. In the latter species he finds the muscles more powerfully developed and their arrangement important for emptying the spleen. In some animals there are even syncytial muscles forming integral parts of the pulp cords.

According to Watzka (1937), one may distinguish species poor in sinuses — cat, dog, cow, horse, sheep, swine, hedgehog, mole, ermine and weasel — from creatures rich in those structures — man, ape, rabbit and squirrel.

Guided by microphotographs from sections impregnated with a silver method, Irma Koboth made a careful reconstruction of the splenic reticulum in 1939. The reticulum she considers identical with praecollagen and resisting the action of chemicals, digestion with pancreatin and necrotizing processes. Its nature and origin are, however, in her opinion, not yet clear.

In her reconstruction of the reticular framework there is shown

a systematic arrangement of the reticular fibrils. The intervals between the annular fibrils vary between 2.6 to 5.2 micra, as measured on her photographs. The annular fibrils anastomose reciprocally, as she found, through filaments of the same size. Every sinus is enclosed by a circle of pulp spaces which is everyone confined by fibrillar fibres departing perpendicularly from the annular fibrils but finer than those. The pulp spaces she calls "Maschengänge" and the system of meshes "Maschengangmantel". Peripherally, these fibres find their anchorage among thick fibres, "Grenzfasern", which envelop within themselves the sinus system as well as the surrounding system of pulp meshes. It is into the "Maschengänge" that the arterial capillaries open and the blood accumulates, while the spleen is functioning as reservoir. These spaces fill, KOBOTH believes, during injection from the artery, just as the spleen sinuses replenish when injecting from the vein.

MOLLIER maintained that endothelium belonged to the reticular tissues, and KOBOTH holds the same view. She points out that it is possible for endothelium to form reticular fibrils as, for instance, is seen in hypernephroma. Pulp reticulum and endothelium are, thus, of the same nature, and belong to the vessel. Whether circulation is regarded as open or closed, that she considers being of minor importance. She thinks that plasma is, already during its passage through the spleen arteries, transmitted to the arterial lymphatic sheaths. The inspissated blood then passes out into the "Maschengänge", whence the reticular cells by their contraction force it through the stomata of the sinus wall. The capillary surface and that of the reticulo-endothelium reacting with blood gets enlarged by coupling to the reticulo-capillary tissue, which is her name for the pulp meshes. These contribute also to the slowing up of the blood stream.

Summing up, it may be said that the elements building up the sinus wall are known with respect to their morphological appearance and topographical relations. The character of the spleen sinus wall as being a perforated membrane can be stated as generally accepted tho KNISELY, however, doubts it. On the other hand, there is nothing known about their functional qualities, the elasticity and contractility of annular fibrils and rod-cells, nor about the variable size of the stomata as encircled by those fibrils and cells.

Pathological Anatomy of the Sinus Wall.

In 1927, LUBARSCH points out the great difficulties one will come across when trying to make a histologic analysis of the minute anatomy of the spleen because of the rapid post-mortal autolysis, to which the spleen surrenders before other organs. This he believes to be the case, especially during septic splenitis. In performing an autopsy in such cases, he found the typical change of the consistency already after $^1/_2$ to 1 hour *post mortem*. He thinks that all delicate structures are very quickly destroyed and that minute vessels, for instance, may be found only in very fresh material.

However, there is a work where these delicate structures have been subjected to a penetrating histologic analysis. This was accomplished already in 1915 by the Japanese MATSUI in a study of the splenic reticular framework in 156 cases, succumbed to various disorders. Using a silver staining method, he was able to demonstrate even minute details of the sinus wall. He considers the filaments of the red pulp so resistant to all sorts of necrotizing processes that they may still be demonstrable in exhumed bodies. As an adherent to the doctrine of closed circulation, MATSUI came through his studies, however, to the conclusion that the sinus wall might, during certain pathological conditions, be loosened up, with extravasations of erythrocytes into the pulp cords as a consequence. That which makes his work right here of special interest, is the fact that he does attach a great importance to the changes taking place in the sinus wall structure. In normal spleens he measured the intervals between the annular fibrils to be somewhere between 4 and 6 micra while the distances between the rod-cells were 1—2 micra. In acute splenitis, acute leukemia and acute splenic congestion, MATSUI finds the sinus wall dilated and loosened up, the intervals between the annular fibrils lengthened, and that the fibrils were at times even torn off. Their silver-affinity MATSUI then finds reduced, their appearance is often rather dimlike. In chronic congestion, chronic splenitis and cirrhosis of the liver, he finds the annular fibrils thicker and the spaces between them more tight because of an increasing number of anastomoses. In chronic leukemias he makes the interesting observation that the structure of the annular fibrils is transformed into a reticular one so that a fine fibrillar net-work surrounds the sinuses.

Another study, which puts the size of the stomata in relation to

the red corpuscles passing through them, was published, in 1941, by WHIPPLE, KNISELY's critical after-investigator. As known, one will in hemolytic jaundice find the pulp cords crowded with red cells and the sinuses compressed. This WHIPPLE considers due to the fact that the erythrocytes being deformed into the shape of spherocytes, meet more obstacles when passing from pulp partitions into sinuses — the open pathway teaching -- than the normal discoid ones. By the pooling of blood in the pulp cords the sinuses are, furthermore, compressed, the stomata turn smaller and, consequently, there is created a viscious circle.

Surveying that part of splenic literature which deals with pathological anatomy of the sinus wall one must say it is a subject very little treated, particularly with respect to the actuality KNISELY's studies have lent to the spleen sinuses. The investigations of Matsui suggest, however, the idea that there may occur changes in the characteristic structure. There is, then, also reason to assume that these changes may act upon the function of the sinus.

Physiological Considerations

Of the many physiological tasks ascribed to the spleen only those two connected with the function of the sinus wall will here be reviewed, viz. the reservoir function and the qualitative alterations of the blood due to it.

The Reservoir Function

The ability of the spleen to change its volume has been known for a long time. Thus, MALPIGHI already thought that the function of the trabeculae was to empty the venous cells in order that the blood should not stagnate and coagulate in the spleen. In 1723, STUKELEY made some experiments in dogs, and by injecting blood into the jugular vein, he found the spleen volume enlarging. He makes the followin penetrating statement: "We make more blood than is just necessary for the occasions of life. Even regular exercise and muscular motion demand a considerable plenitude of juices and if there was not more than enough we should be continually fainting." STUKELEY regarded the spleen as a storing place for a surplus of blood, which could, in case of need, be used. In 1830, DOBSON found the spleen to expand during

digestion. He believed that an access of blood during it ensued and was gathered in the spleen. He repeated the experiments of Stukeley and arrived at the same results.

In 1923—24, Barcroft and co-workers made experiments on rats in order to verify some occasional findings concerning the variations of the circulating blood volume. They call attention to the fact that the blood in the rat-spleen can temporarily be shut off from circulation. Altho the rats inhaled carbon monoxide, yet their splenic blood was found kept clear from it provided they were at rest. This difference between peripheral and splenic blood could not be ascertained during muscular stress. Later, in 1927, Barcroft and Poole showed that the blood shut off from circulation in the pulp had a far higher concentration of hemoglobin than the peripheral blood. This was demonstrated on a dog whose spleen was exteriorized subcutaneously in order to study its variations in size. It appeared that certain irritations provoked a contraction of the spleen, especially muscular strain and loss of blood, but also some purely psychic stimuli, for instance, such a one as when the dog caught sight of a cat. This was reflected in the peripheral blood as a temporary rise of hemoglobin concentration and the relative number of red cells. The property common to all irritants capable of producing a splenic contraction Barcroft and Nisimaru discovered, in 1932, to be their ability of provoking a rise in the arterial blood pressure.

Thanks to the investigations of Barcroft, we have this fact about the splenic reservoir function, now regarded as the most significant chapter in the physiology of the spleen. It has also brought about important consequences for the clinic.

Contemporary with Barcroft, Binet and his fellow-workers in France studied the function of the spleen as a reservoir organ. In 1930, Binet published his results indicating that acute asphyxia, produced by pinching the trachea, in dogs mobilized the reservoir blood. By comparing this reaction in splenectomized dogs and others not, he could reach some conception as to the capacity of the splenic reservoir. During contraction of the spleen he found twice as many red corpuscles in the splenic vein as in the artery. In the peripheral blood the number of erythrocytes rose with 1—2 millions per cmm^3. In cats the rates were somewhat lower, in horses higher. The reaction disappeared when the spleen stylus was tied off. In splenectomized animals it could not at all be provoked. Binet thinks, moreover,

he is able to prove that there also in man is a storing function; for in a woman whose spleen was easily felt, he found, after muscular work, an increase in the number of erythrocytes simultaneously with a contraction of the spleen. In order to elucidate how the concentration of red cells is effected in the spleen, BARCROFT and FLOREY (1932) made experiments on cats by way of tying their splenic veins. They found, nevertheless, an actual concentration of erythrocytes taking place. This they thought impossible if the blood fluid had escaped through the vein while the red corpuscles remained in the pulp. Having observed how a flow of blood-stained lymph, during the experiment, whirled into the *cisterna chyli*, they concluded that plasma escaped by lymphatics. This view may, however, be challenged, because every histologist working on this subject has, since 1900, denied the existence of deep lymphatics in the spleen. Besides that maximal contraction of the spleen, as the mobilization of the reservoir blood implies, many investigators (ROY 1881, HARGIS & MANN 1925, BARCROFT & NISIMARU 1932, GRINDLAY, HERRICK etc 1939) have observed rhythmic variations of minor amplitude occurring at regular intervals. These rhythmic contractions of the spleen are still unknown with respect to their causes and anatomical basis.

HAHN, BALE and BONNER estimated, in 1942, the volume of circulating red cells in dogs by means of radioactive phosphorus as well as with hematocrit. Their estimates were performed before and after injecting epinephrin intravenously. They subsequently found an increase in the hematocrit values. This was, however, not the case in splenectomized dogs. Neither in them nor in others did there appear any rises in the volume of the red cells as measured by way of labeled ones. The reason why, they cannot explain.

Using the same technic (P 32) HAHN and his collaborators could, nevertheless, demonstrate a decrease in the number of circulating erythrocytes in dogs narcotized with nembutal. The reduction amounted to 37 %. In splenectomized dogs they, however, registered a far smaller decrease, viz, that of 15 %. By making use of the hematocrit method in the same year (1942) on dogs under nembutal narcosis, JARCHO got similar results.

The findings in the nembutal experiments indicate that during certain conditions there may exist an intensified storing function. The occurrence of such a phenomenon during digestion was already observed by DOBSON. His find has been substantiated by many later

investigators, the last of them being KNISELY, who found an increase in the number of storing sinuses after feeding. In pregnancy there appears also a testified splenomegaly. Such an increased reservoir function might essentially be the very cause of the acute splenic swelling, as it turns up under various circumstances. In acute infectious diseases the red cells will accumulate in the splenic pulp. This several investigators (KLÖNE 1940, GÜNTHER 1940) have experimentally shown. RICH (1928) found that acute splenic swelling was not only dependent on a bacteremia but could also be produced by injecting foreign protein. The cause of it he believes to be an antigen-antibody reaction.

Even upon intoxications with blood poisons one will run across an acute splenic hyperemia simultaneously with the decrease in the values of hemoglobin and erythrocytes (JAWEIN 1900). It is, above all, poisons producing alterations in the red cells which are apt to lead up to such acute tumefactions of the spleen. In 1901, HEINZ regards the spleen as a storing place for these effete erythrocytes, "Bluttrümmern." Due to their characteristic shape, he could directly identify them in the spleen sinuses.

Qualitative Alterations in the Red Cells during the Reservoir Function

In 1849, ALBERT VON KÖLLIKER, who has already been quoted, advanced the theory that the splenic function is to destroy erythrocytes. As a proof of this he cites microscopic observations showing that during aggregation of red cells, occurring in the spleen, their cellular borders got lost.

His theory soon got support from GRAY who, in 1854, studied the splenic venous blood and compared it with blood from other vessel-areas. The investigations were carried out on horses. He found that the venous blood from the spleen contained fewer erythrocytes but a higher amount of fibrin than peripheral blood. Most interesting are his observations about the altered shape of the red cells in the splenic vein. He states they lose their biconcavity and decrease in size. His description suggests that he has observed discoid red cells transformed into spherocytes. Serum showed, he found, a dark reddish tinge. From these and other observations he concluded that the spleen really destroys red cells.

The question about splenic hemolysis has, subsequently, not ad-

vanced to any considerable extent. Reviewing present investigation results, LAUDA points out in a monograph on the physiology of the spleen in 1933 that they lend no support to the idea that the spleen should be hemolytically active.

FÅHRÆUS (1921) — later together with BERGENHEM (1936) — studied the changes that blood is subjected to when kept in vitro at body-temperature. A blood specimen with a high sedimentation rate, then, shows a decrease in its sedimentation velocity. The explanation for this alteration should, according to FÅHRÆUS, be sought in changes taking place partly in red corpuscles and partly in the blood fluid. At the same time as the sedimentation rate decreases the erythrocytes show a conversion of their discoid shape into a spheroidal one. The rouleaux-formation is simultaneously diminishing. As the cause for this conversion of the red corpuscles attributable to the blood fluid BERGENHEM discovered the action of lysolecithin. This was, in turn, manufactured through the action of a lecithinas upon the lecithin in the serum.

Trying to transmit this *in vitro*-acting mechanism to a physiological process *in vivo*, FÅHRÆUS and BERGENHEM at first pointed out that an analogous process must be conceived of occurring in the spleen where the blood can, as shown by BARCROFT, be shut off from circulation. Their experiments seemed to show a stagnation of the blood to be essential. For this stagnation in the spleen and the conversions of the blood taking place during it they coined the term "endopaus" of the blood. Later, in 1938—39, it became evident to BERGENHEM and FÅHRÆUS that it was the reduction of the boundary surface between erythrocytes and plasma, appearing during the stagnation which brought about an increment in the suspension stability of the blood. The mechanical factor consisted, in other words, of a separation of plasma from the red corpuscles. KNISELY's observations, then published, suggested a filtration of plasma taking place through the sinus wall during the filling phase, and this observation enhanced the earlier assumption of the authors that the function of the spleen really is to produce a change in the blood quality.

FÅHRÆUS (1939) calls attention to older observations about an alternation in the shape of red cells in the splenic venous blood indicating a greater number of spherocytes and a diminished rouleaux-formation as compared with that from other vascular areas. In 1939, MELLGREN pointed out alterations in the qualities of the reservoir blood in the cat-

spleen. He found this very blood manifesting a higher degree of erythrocytic concentration as well as an abated aggregation tendency. Moreover, he noticed that plasma from the reservoir blood contained some dissolved hemoglobin indicating a partial hemolysis, occurring in the blood during its capture in the spleen. In 1940, NORDLANDER showed that the storing blood from the cat-spleen had in its plasma a higher standard of potassium. This finding he interpreted in favor of hemolytic processes.

KNISELY believes that the purpose of the peculiar activity of the spleen sinuses is "to act upon blood." MCKENZIE and associates held, however, that the red cells in the spleen are destroyed by means of erythro-fagocytosis in the pulp cords, which they were able to see in the microscope. There they also found evidence of deplasmatization *ad modum* FÅHRÆUS. As a third possibility for bringing about hemolysis they regard the dangerous wear and tear of red corpuscles in the narrow channels of the pulp cords.

In 1941, HAM and CASTLE found that a stasis, as well *in vitro* as *in vivo*, caused an increase in hemolysis. Quoting KNISELY's work, they point out the congestion normally appearing in the spleen, and believe that splenic hemolysis is brought about through erythro-stasis.

Summarizing, one may conclude that there is a reservoir function for the red cells in the spleen, at least for some animal species. While reviewing this property of concentrating erythrocytes from the standpoint of earlier mentioned circulation theories, we arrive at a certain appreciation of their validity.

The closed circulation, teaching that the blood passes only through the red pulp in a closed vascular system cannot cause a concentration of erythrocytes. The open circulation may, on the other hand, give rise to such a concentration in the manner outlined by MCKENZIE and co-workers through randomly recurring storing in certain pulp meshes. It is, however, rather disagreeable to postulate that a physiologic function should for its existence be dependent on a causal stoppage of the circulation through aggregated white corpuscles.

The circulatory pattern that in the best possible way satisfies the claims the storing function lays on the shape of the vessel pathway, is, beyond doubt, the one here designated as the divided circulation. In this way, the concentration of red cells, as regulated through the cyclical sinus activity, will receive a natural explanation. Simultaneously, the findings of BERGENHEM and FÅHRÆUS obtain their physio-

logical background and meaning. KNISELY could directly observe how erythrocytes were deplasmatized while staying in the sinuses i. e., under the influence of the reservoir function. This process evidently implies a conversion of the qualities of the blood. And, what is more, the very same changes may be brought about *in vitro* by deplasmatizing red corpuscles.

II. The Problem

From the review of literature above it is obvious that there are three fundamentally different alternatives about the splenic circulation, viz. the theories of the open, closed and divided pathway. There is, then, first of all, the doctrine of the open one stating that the sheathed capillaries pour their blood into the pulp cords from where it is, subsequently, forced through the sinus wall into the sinuses. Secondly, the theory of the closed circulation. Its adherents declare that the blood, as in other organs, travels everywhere within a closed vessel-system. Finally, one will, in the third place, have to consider the thesis of the divided circulation. This proposes that a separation of red cells from plasma is the essential feature in the splenic circulation.

He who reads the works of KNISELY cannot get rid of the impression that this investigator has, in his exact observations and well justified interpretation of them, found a convincing explanation of the spleen sinuses as constituting a filtering mechanism. This impression is, moreover, but strongly fortified when investigators, like FÅHRÆUS and BERGENHEM, who in their work proceed from quite different starting-points report results pointing to the fact that the very changes one may expect to take place within the spleen, are *in vitro* really brought about in erythrocytes as made devoid of their blood fluid. However, it is evident that KNISELY's view has not been accredited, at least, not widely accepted. After-examiners have, as seen above, tried his methods but without being able to confirm his findings. They take, on the contrary, a position in favor of the open pathway theory. Modern histological textbooks persist in this teaching while at the same time declaring that the problem of splenic circulation is not yet solved.

In this situation, it is obvious that there must be a vital point lying in the anatomical properties of the sinus wall regarded as a filter or, more precisely, in estimating the size of its pores. The structure of the sinus wall is well-known. Highly characteristic is its nature of being a fenestrated membrane. Then, it is a matter of course that the degree of permeability must vary with the size of these pores, stomata. The problem of estimating this size and its eventual variations, then, arises.

Intimately connected with this problem is the question about the circulation type. Does there exist a stream through the sinus wall? If so, which direction does it take? Does it travel from the sinuses into the pulp cords or vice versa? If the sinus wall is a filter, what course does the filtration take? To answer these questions is also to solve the old problem of circulation, so essential to the spleen.

III. Animal Experiments

The Red Corpuscles and the Sinus Wall

All histologists, no matter which pathway they have advocated, are agreed upon the fact that one will always find erythrocytes in pulp partitions; and when all is considered, it must be this finding which is responsible for the setting forth of the open circulation theory. In a second work, published in 1936, KNISELY explains how the pulp cords get crowded with red cells. While transilluminating the spleen, he found this take place *in agone*. Then — as even after mechanical irritation — he saw plenty of red corpuscles permeating the sinus wall in order to enter the pulp partitions. At first, the erythrocytes got deformed while passing through the wall; subsequently, they traveled apparently without meeting any resistance. It seemed to Knisely as tho the permeability of the wall had suddenly changed. Any considerable alteration in the volume of sinuses he was unable to watch. In the living spleen showing this reaction to mechanical stimuli, the red cells returned into the sinuses after irritation ceased. In the dead organ all sinuses are of the same average width which is never the case in the living one. KNISELY assumes that these dramatic phenomena, so suddenly arising, are sufficient to explain why one will, in fixed material, never meet with those peculiar sinus pictures altogether characteristic in the living animal. The changes, as related above, take place, of course, much more rapidly than even the fastest fixation aid can penetrate the tissue.

This finding about the sinus reaction corresponds very well to observations of earlier investigators who, having used the injection method, discussed extravasations or open circulation. It was the very withdrawal of erythrocytes that KNISELY was able to watch. His supposition that an agonal alteration in the permeability of the sinus wall should be the cause of this phenomenon, did feed the idea, that by changing the size of the red corpuscles, one could possibly

prevent them from forcing their way out through the wall. For the purpose of bringing about such a change in the red cells two different methods are at our disposal.

Rabbits have, for many reasons, been chosen as experimental animals in all my investigations. Only in man, ape, dog and rabbit the sinus wall consists of a regular network, according to MOLLIER, as already seen. In the opinion of WATZKA, man and rabbit belong to the very group of creatures which have a richly developed sinusoidal system. Since the results collected from my animal experiments will, in part, be correlated to findings from human autopsy material, it is important that the examined specimens show a structure so similar to that as possible. Nor are rabbits, like rats and mice, subjected to chronic infectious diseases (*Bartonella*), which cause alterations in both structure and function of the spleen.

Injection of Gelatin

In 1921, FÅHRÆUS pointed out that erythrocytes get aggregated into rouleaux-formations by the presence of highly viscous hydrophile colloids, as, for instance, gelatin. This takes place both *in vitro* and *in vivo*. For the aggregated red cells it ought to be more laborious than for those others which appear single-handed to squeeze through the stomata in the sinus wall, even if these were enlarged. Proceeding on such argumentation as a basis, I injected some rabbits with 20 ml 10 % gelatin at a temperature of 37° C. intravenously. The sedimentation rate rose instantly from ca. 2 mm/hour to ca. 130 mm/hour. It was easy to observe in the sedimentation tube that a strong aggregation took place among the red corpuscles for the column of blood had a granular appearance. As FÅHRÆUS has shown, an increment in the rouleaux-formation can also be demonstrated *in vivo*.

The injection effect upon the splenic structure was very striking. In spleens from untreated rabbits there occurs a diffuse distribution of erythrocytes, just as many in the pulp partitions as in the sinuses. But this is not the case in spleens from rabbits injected with gelatin. See fig. 2 and 3. Almost all red cells are here situated in the sinuses; only occasionally, one runs across smaller pools of blood in the pulp cords. The boundary-line between sinuses and pulp partitions gets clearly visible. It was, in other words, evident that the injection of gelatin had reduced the crowding of the pulp cords with red cells, other-

wise regularly recurring *in agone*, as observed by KNISELY. This result is inexplicable from the view-point of the open circulation, according to which the aggregations of the red cells should have taken place in the pulp cords. These would, then, consequently have increased in size because the passage of the cellular aggregations into the sinuses would have been blocked. By diminishing the filterability of blood[1], i. e. by changing its suspension degree into a coarser one, one will bring about an alteration in the splenic structure showing that the open pathway theory is improbable. So the truth must be sought in either of the other two alternatives — the closed or the divided circulation theory.

Injection of Saponin

By making aggregations of erythrocytes, one could thus, to some extent, avoid crowding of the pulp cords with red cells or, as it were, fix their site within the vessels. This awakened the interest about the problem: What effect does a change in the shape of the red cells produce? When scrutinizing the possibilities open to a red cell to leave the spleen sinus through a stoma in the wall it stands out clearly that it may pass trough such a slit having the same measures on its boundary as itself, i. e. for the rabbit 6,9 × 1,9 micra. If it is, for instance, subjected to a filtration pressure, as must be the case in the supposed filtering-filling phase of KNISELY, then it may even squeeze through smaller slits, according to its elastic properties, i. e. its capability of getting deformed. The filtration pressure must, then, overcome the forces inside the red corpuscle aiming to retain its discoidal form. From the results KNISELY obtained, one may conclude either that filtration pressure rises in agone or that the size of the slits increases at that moment.

It is known that by adding weak hemolytics one can transform the biconcavity of the red cell into a spheroidal shape. As a consequence, it follows that the erythrocyte gets a diameter common for all planes; its shortest diameter increasing and its longest decreasing. This may

[1] A parallel to the filtration phenomenon, here indicated, is probably the so called "Guttadiaphot"-test, introduced by SCHILLING, in 1928, as a clinical investigation method. It is based on the fact that whole-blood when dropped on a filter-paper in various disorders gets encircled by a "corona" of various breadths. This corona consists of a plasma-ring filtering out from the drop-spot and being, in relation to its center, poor in erythrocytes. Same diseases which cause a broad corona also cause a high sedimentation rate.

diminish the possibility for red cells to pass through the wall, N.B., provided the slits are not spherical. Right here our attention is called to another fact. Certain observations made on spherical red cells (FÅHRÆUS, 1946) indicate that their elasticity, as compared with discoid erythrocytes, is minimized. Such a decrease in the elasticity must omit the chances for the cell to squeeze through a stoma narrower than its diameter.

Tests made on rabbit erythrocytes *in vitro* revealed that by adding 33 mg of saponin to 100 ml blood one could produce spherocytosis without bringing about hemolysis. The blood volume of the rabbit was estimated to a 1/13 of body weight. Proportionally, saponin in a solution of 0,25 % was injected intravenously and, then, the rabbits were killed. While the animals seemed to withstand the injection of gelatin quite well, they appeared ill after the injection of saponin, and some had loose stools.

The effect upon the splenic structure was, however, of the same kind as in gelatin experiments. The pulp cords are distinguished by their relative freedom from red cells. As usual, however, the amount of blood in the pulp partitions is greater in the saponin experiments than in the gelatin ones. It is, nevertheless, evident that simply through a transformation of the red cell and perhaps even of its elasticity — a change within the range of a few micra — it will be possible to reduce the erythrocyte-loading of the pulp cords. This result, too, must be interpreted as caused by a lessened filterability of the blood suspension.

Summing up these experiments with gelatin and saponin, one may say that the site of the red cells in living spleens, is intravascular. Any hint as to whether the circulation bears the character of being closed or divided they do not give. It is obvious that small changes in the size of the suspended particles are important for its filterability. On account of this, there turned up the interesting problem to estimate the size of the stomata in the spleen sinus wall of the living rabbit.

Attempts to Use an Indirect Method of Measuring the Size of the Stomata, in the Spleen Sinus Wall

The outline for this part of my investigation has been to inject rabbits with a suspension containing particles of varying size. By means of these particles it would be possible to ascertain partly the

width of the stomata and partly the direction of an eventual stream through the sinus wall, provided that such a one could be shown to exist. In closed circulation all grains ought to lie intravascularly. If the bigger grains were refound in the pulp cords and the smaller ones inside the sinuses, this would point to an open circulation. A reverse order, i. e. the bigger in the sinuses and the smaller in the pulp partitions would indicate the existence of a divided circulation. For the purpose of securing as physiological conditions as possible, there should pass a certain time from the injection of the suspension to the killing of the animal in order to achieve that the grains would be deposited under undisturbed circulatory terms. Finally, the experiences gained in previous trials should be used to facilitate the determination of the site of the grains by fixing the vital position of the red cells through gelatin or saponin injections.

Rice-starch, suspended in physiologic saline, has been used as a testing suspension. It is the starch specimen that has the smallest size of its grains. To avoid the occurrence of too large a number of distressing embolies it was necessary to get rid of bigger grains or conglomerates of such by passing the starch through an apparatus, especially constructed for this purpose. It was built up of five glass-columns coupled in series with a volume of ca. 400 ml each. They have an increasing wideness from 3 to 12 cm in diameter and are run through with water from beneath. See figure below.

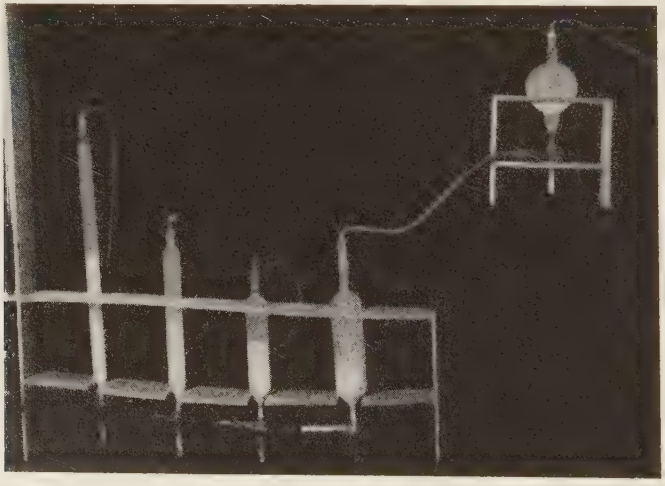

Fig. 1.

The velocity of the water-flow is decreasing in proportion as the width of the columns swells out. Consequently, the biggest grains and conglomerates settle in the first and the smallest in the last column. Using a stream-velocity of ca. 40 ml/min, I have found the grain size in last column to be:

$$M = 4.28 \pm 0.06\ \mu$$
$$n = 324$$
$$\sigma = 1.29 \pm 0.05$$

This sediment has, then, been suspended in ca. 10 parts of physiologic saline and 10—20 ml of this suspension were injected into an ear-vein of the animal. The rabbit was then left in piece for an hour in a dark box. Rabbits seem to withstand the rice-starch injection quite well and may, subsequently, live several days in apparently good health. In the box they sit in rabbitical tranquility. After ca. an hour 20 ml of a 10 % solution of gelatin at 37° C. or advisable amount of saponin in a solution containing 0.25 % was injected intravenously in order to prevent the red cells from leaving their vital traveling pathways. The rabbit was then immediately killed either by air injected intravenously or, in the latter part of the experimental series, by sodium amytal in order to prevent the agonal seizures. As soon as the animal was dead, its abdomen was opened and the spleen excised, divided into two equal parts and immersed into a glass-receiver containing methyl alcohol. Slices of liver, lung and bone marrow were, then, cut out. The liver was examined with respect to the occurrence of coccoidal granuloma which was a rather rare find. The reason why methyl alcohol was used as a fixation aid depends on the fact that I in some previous experiments incidentally happened to use it and then found it to be a very good fixation aid, at least for splenic material. Its shrinking effect is not noticeable. It appeared altogether superior to formol, and delivers nicer sections with a better differentiation of the staining. The cause of this is not clear and has not been penetrated any further.

The collected slices were then treated, according to prevailing histologic methods. Yet it was necessary to use a workable technic without heating in order to avoid that the injected starch-grains should gelify[1]

[1] According to chemical handbooks starch gelifies at temperatures above 60° C. A comparison between the appearance of the starch-suspension before and after its injection indicates that there did not occur any alteration in the shape of the rice-grains.

at higher temperatures and change size. Celloidin embedding proved better than the freezing method. Section-thickness varied between 7 and 10 micra. Staining with iodine and using "Kernechtrot" as a contrasting stain, I found the iodine-staining to give its best results when some crystals of it were added to the last xylol before mounting. The dark blue tinged starch-grains as found in freshly stained sections otherwise show a tendency to fade. "Kernechtrot" permits a rather good differentiation between various details.

Using this method, I examined 71 rabbits. The density of the refound starch-grains is always greatest in sections from the lungs. There several of them had, as it seemed, been entrapped in the capillaries. Liver and spleen came next in density while the bone marrow contained exceedingly few grains. In the spleen, their number is always largest in the pulp cords where they usually are found keeping clear of the pulp cells. Some, but certainly not many, grains lie in the few existing pulp capillaries. Any difficulty to distinguish pulp partitions and sinuses from Malpighian corpuscles does not arise. Likewise, one discerns clearly the boundary between pulp cords and sinuses.

It is not at all difficult to decide the position of grains lying in open sinuses. There does not seem to be any gathering of them at the sinus wall, they are on the contrary found everywhere in the sinus lumen. Those grains localized in the pulp cords may, however, raise difficulties. Particularly in spleens crowded with red cells it may be a hard task to decide if a pulp-grain lies free among the pulp-cells, in the periphery of a tangential cut sinus or in the lumen of a contracted sinus with an imperceptible lumen. Above all, these difficulties are great when the pulp cord shows a breadth rising to 20—30 micra.

The diameter of the starch-grains I have measured with a micrometer ocular at a magnification rising to 1000 ×. The grains appear in the sections as slightly irregular spheres, sometimes, however, more or less elliptical. Hence it was necessary, even from the very start, to lay down certain rules for measuring the diameters of the grains. The slide was moved forward by means of a crossboard at right angles to the scale mounted in the ocular. When a grain touched the scale-lines, its breadth was always measured, as it appeared and without turning the micrometer ocular. Some irregular grains were, consequently, measured to a greater breadth than their smallest one.

Due to the difficulty in deciding the real site of those grains lying

in the pulp cords, a certain rule has, furthermore, been applied, viz. only to measure those grains situated within a distance of 5 micra from a distinctly visible sinus wall. These granules could fairly well be observed as located freely within pulp spaces provided they did not appear in the capillary lumina. The number of grains which could, according to this rule, not be determined was larger than that which could be located.

It must happen, as a matter of course, that some grains during sectioning got divided into parts with unequal size. This mutilation will, however, to the same extent hit the pulp-grains as the sinus-grains. Granules having a low staining intensity or other clear signs showing their nature of severed splinters have not been measured. All measurements have been made by the author himself and this gives a certain guarantee for a uniform estimation.

An experiment showing that the location of grains in the spleen has taken place during life is the following. Having received an injection of starch suspension a rabbit was after the elapse of an hour exsanguinated. Its blood was then transfused into another rabbit. In its organs no starch grains were found upon death and so it is evident that the blood of the "donor" must have been practically free from starch grains. The organs of the "donor" were, however, full of them. These grains, especially in the spleen, had, therefore, found their place there prior to the killing of the animal.

Results:

The starch granules have, in connection with their measurements, been divided into three groups under these covers: sinus-grains, pulp-grains and grains of uncertain site. For each group a mean has been calculated on the basis of values found in the 71 normal rabbits examined.

Even other statistical data have been calculated according to prevailing methods.

The mean size for the sinus width in the 71 normal rabbits rises to: $M = 27.72 \pm 0.21\ \mu$, $n = 3596$, $\sigma = 12.50 \pm 0.15$.

The mean size for all the grains found in the sinuses: $M = 3.51 \pm \pm 0.02\ \mu$, $n = 1789$, $\sigma = 0.90 \pm 0.02$.

The mean size for all grains found in the pulp cords within a distance of 5 micra from the sinus wall: $M = 2.70 \pm 0.01\ \mu$, $n = 2914$, $\sigma = 0.75 \pm 0.01$.

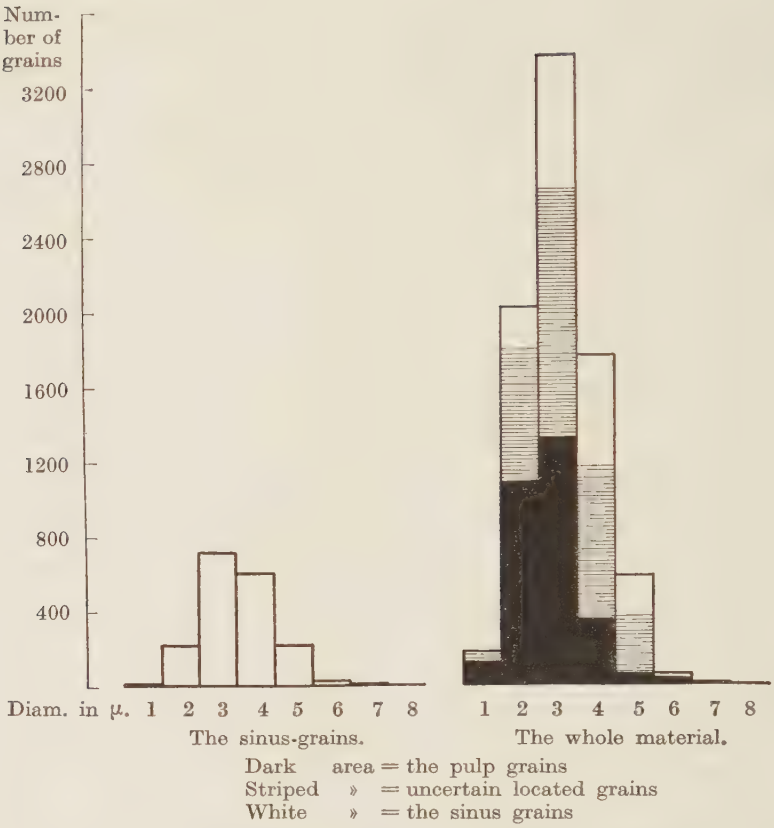

Diagram 1. The distribution of the size of the grains in normal rabbit group.

The mean size for grains with uncertain site in the spleen:
$M = 3.23 \pm 0.02\,\mu$, $n = 3246$, $\sigma = 0.98$.

The mean size for the sinus- and pulp-grains:
$M = 3.01 \pm 0.01\,\mu$, $n = 4703$, $\sigma = 0.90 \pm 0.01$.

The mean size for the whole material of splenic grains:
$M = 3.10 \pm 0.01\,\mu$, $n = 7949$, $\sigma = 0.97 \pm 0.01$.

The difference between the means for the size of sinus-grains and the pulp-grains rises to: 0.81 ± 0.02. It is statistically significant.

The distribution of the total mass of starch grains shows a skewness amounting to -2.8 ± 0.34 (calculated according to LINDEBERG). The grains which have an uncertain localisation show a more normal

distribution with a skewness only rising to —1.9 ± 0.53. This fraction is, however, to a greater extent derived from the bigger grains than from the smaller of the total mass, and that is, for instance, evident from the fact that the mean for these grains is higher than that for the whole material. The difference rises to: 0.13 ± 0.02. Because of that the skewness for the rest of the material, i. e. the sinus- and pulp-grains, is not very pronounced, only rising to —2.0 ± ± 0.44 with a mean which reaches 3.01 ± 0.01 (for the whole material 3.10 ± 0.01). Finally, in surveying the sinus- and pulp-grains, one finds that, above all, small grains are found in the pulp cords. Of the granules measuring 1 μ in width 95 % are refound in the pulp cords and only 5 % in the sinuses. Those measuring a diameter of 5 μ are only to 19 % located in the pulp cords and, consequently, to 81 % in the sinuses. According to this fact, there arises a normal distribution of the pulp-grains; the skewness only amounting to 0.5 ± ± 0.56. The sinus-grains show, however, a greater skewness. From the total distribution there has been subtracted the mass of pulp-grains, which show a practically normal distribution. Because of that the skewness of the sinus-grains increases, viz. —2.4 ± 0.71.

The reason for the skewness of the grains found in the spleen should be sought in the fact that the starch suspension has been filtered through passing the lung capillaries.

The result of the measurements signifies that the injected starch suspension has been distributed, according to the size of the particles. The mean size of the sinus-grains is greater than the mean size of the pulp-grains. It is, in other words, evident that the sinus wall constitutes a boundary between larger and smaller granules. The explanation can hardly be anything but that the spleen sinus wall has acted as a filter retaining the larger particles. The mean size of the pulp-grains must then be influenced by the size of the stomata in the wall.

This experiment confirms the results already indicated by the effect of injected gelatin and saponin, viz. that circulation for red cells is closed. It goes, however, a bit further. The filtering function of the sinus wall, as demonstrated, is obviously the same thing that KNISELY could show in his experiments during transillumination. There takes place a separation through the sinus wall which retains larger particles within the sinuses while smaller ones and, of course, also plasma escape into the pulp cords. If there had existed an open circulation, then the distribution of the granules would have been the opposite and the

pulp partitions would have retained the larger particles. Thus, only the smaller ones would have had an open access into the sinuses. This type of circulation can, therefore, be ruled out. Likewise that of the closed pathway because there is no closed circulation inasmuch as a part of the circulating matter escapes from the vessel-pathway. By way of conclusion there must be a divided circulation in the splenic pulp, according to the pattern vindicated by KNISELY.

This argumentation is based on the assumption that gelatin and saponin *per se* have no direct effect upon the sinus wall but that the result is produced by their acting upon the form and aggregation tendency of the red cells. Five experiments made on rabbits which were not treated with gelatin or saponin previous to death, show, nevertheless, the same principal range of size as the treated ones, altho the measurements were more difficult due to the presence of red cells in the pulp cords.

These experiments gave following values:

Sinus-grains: $M = 3.38 \pm 0.08\,\mu$, $n = 138$, $\sigma = 0.91 \pm 0.05$.
Pulp-grains: $M = 2.80 \pm 0.03$, $n = 514$, $\sigma = 0.80 \pm 0.03$.

The difference here runs in the same direction.

Attempts at Estimating the Size of the Stomata in the Sinus-wall in Experimental Infections and Intoxication with Hemolytic Poison

The finding of a filtering function of the spleen sinus wall in normal rabbits does induce an investigation of this function in animals during pathological conditions producing acute splenic swelling. In order to bring about such a one, I used, in the following experiments partly infection with *streptococci* and partly poisoning with phenylhydrazin hydrochloride.

The infected group consisted of 32 rabbits. They were inoculated with 0.1—0.3 ml of a 24 hours' old broth of β-hemolytic *streptococci* intracutaneously in the ear. 24 hours later, I made a test with rice starch suspension. The procedure was exactly the same as previously used in normal rabbits when the method of injecting gelatin was applied in order to prevent the red cells from leaving their vital pathways *in agone*. The effect of the inoculation was, in general, quite noticeable after 24 hours; the rabbits appeared ill and would not feed.

In some cases one could culture β-hemolytic *streptococci* from the excised spleen. A bacteriologic control was, however, not used as a routine.

The other group consisted of 20 rabbits, injected with 2.5 cg phenylhydrazin hydrochloride pro kg body-weight subcutaneously. This poison constitutes a rapidly working hemolyticum. In my experiments the dose, mentioned above, brought on a decrease in hemoglobin values and number of red cells pro cmm^3 to half of the original values. Contrary to the group of infected animals, these rabbits seemed to be quite well. After 24 hours they were subjected to an injection of rice starch suspension in the same way as the foregoing group.

At autopsy there was, on the whole, an enlarged spleen observable in the infected group while it was a rule in the intoxicated one.

Results:

The mean size for the sinus width rises to:

Infected group: $M = 28.27 \pm 0.24\,\mu$, $n = 2188$, $\sigma = 11.25 \pm 0.17$.
Poisoned group: $M = 34.74 \pm 0.34\,\mu$, $n = 1284$, $\sigma = 12.20 \pm 0.24$.

The mean size for grains with uncertain site in the spleen:

Infected group: $M = 3.54 \pm 0.02\,\mu$, $n = 1572$, $\sigma = 0.90 \pm 0.02$.
Poisoned group: $M = 3.55 \pm 0.03\,\mu$, $n = 1050$, $\sigma = 0.83 \pm 0.02$.

The mean size for all grains found in the sinuses:

Infected group: $M = 3.60 \pm 0.03\,\mu$, $n = 1156$, $\sigma = 0.90 \pm 0.02$.
Poisoned group: $M = 3.66 \pm 0.03\,\mu$, $n = 667$, $\sigma = 0.79 \pm 0.02$.

The mean size for all grains found in the pulp cords:

Infected group: $M = 3.21 \pm 0.02\,\mu$, $n = 1820$, $\sigma = 0.89 \pm 0.02$.
Poisoned group: $M = 3.44 \pm 0.02\,\mu$, $n = 1154$, $\sigma = 0.83 \pm 0.02$.

The mean size for the sinus- and pulp-grains:

Infected group: $M = 3.36 \pm 0.02$ $n = 2976$, $\sigma = 0.91 \pm 0.01$.
Poisoned group: $M = 3.52 \pm 0.02$ $n = 1821$, $\sigma = 0.82 \pm 0.01$.

The mean size for the whole material of splenic grains:

Infected group: $M = 3.42 \pm 0.02$ $n = 4548$ $\sigma = 0.91 \pm 0.01$.
Poisoned group: $M = 3.54 \pm 0.02$ $n = 2871$ $\sigma = 0.82 \pm 0.01$.

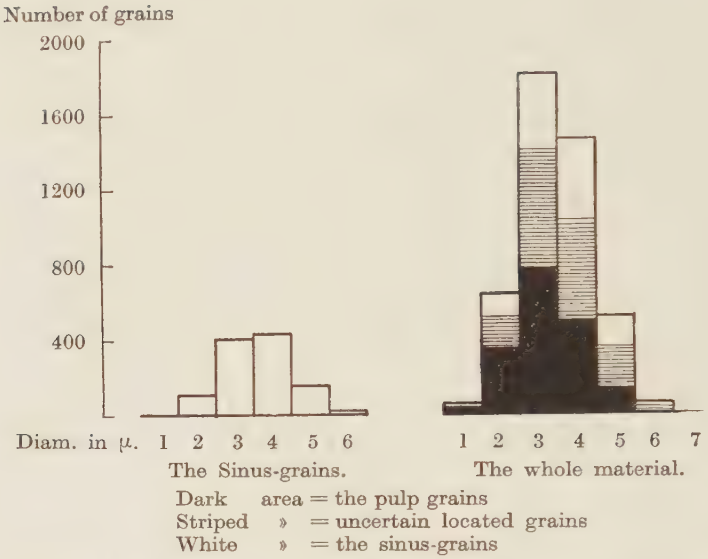

Diagram 2. The distribution of the size of the grains in infected rabbit group.

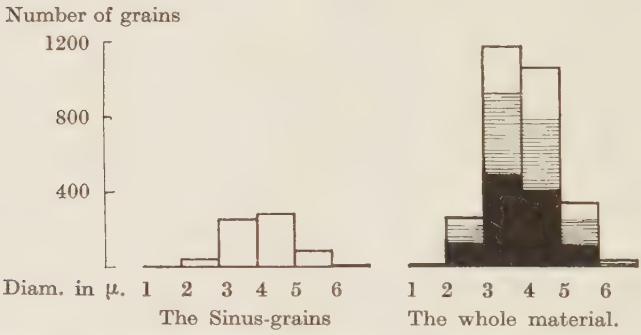

Diagram 3. The distribution of the size of the grains in poisoned rabbit group.

The difference between the mean size for the sinus-grains and the pulp-grains rises to:

Infected group: $D = 0.39 \pm 0.04\ \mu$.
Infected group: $D = 0.22 \pm 0.03\ \mu$.

The distribution of the size of the grains is shown in the diagrams above.

In the rabbits infected with *streptococci* the whole material of grains shows a skewness rising to -1.92 ± 0.44, while in those gra-

nules the site of which could not be determined there is a skewness amounting to —2.77 ± 0.76 as caused by the fact that they are mostly derived from grains with a greater diameter. The mean for this group is, therefore, higher than the mean for the whole material (3.54 ± 0.02 respectively 3.42 ± 0.02 μ). The difference is 0.12 ± 0.03. The mean for the rest of the material, i. e. the sinus and pulp grains, has a lower value, viz. 3.36 ± 0.02 μ, showing a skewness rising to —1.44 ± 0.55. Comparing these grains, one finds — as was the case among the normal rabbits — a higher frequency of smaller particles in the pulp cords. Thus, grains with a diameter of 1 μ to 80 % are located in the pulp cords, and so only 20 % of them in the sinuses. This is shown in diagram 4. While the size of the granules is increasing, their chances to escape into the pulp partitions are lessened but not to the same extent as in the group of normal rabbits. Hence, of the grains having a diameter of 5 μ, still 47 % are found in the pulp cords. The mean size for the pulp-grains is, according to this, higher than that in normal rabbits (3.21 respectively 2.70 μ). The difference is 0.51 ± ± 0.03.

As the chances for larger granules to enter the pulp partitions in this group are greater, the distribution of the whole material and its pulp part shows a greater similarity than in normal rabbits. Thus, the pulp-grains demonstrate a skewness of —1.69 ± 0.70. When this rather skew distribution is subtracted from the that of the whole material which, too, reveals a skew distribution, this results in a normal distribution of the sinus-grains. Their skewness amounts only to +0.32 ± 0.88.

The rabbits poisoned with phenylhydrazin hydrochloride show principally the same distribution as the infected animals. The skewness of the whole material rises to —1.80 ± 0.56. Granules with uncertain location present a skewness of the same range, viz. —1.89 ± 0.93; and the mean size for each of these groups is, too, almost identical (3.54 ± 0.02 respectively 3.55 ± 0.03 μ). Even the pulp-grains have a resembling skewness (—1.78 ± 0.88); the mean for this group is, however, somewhat lower or 3.44 ± 0.03 μ. It must depend on the fact that this group contains a bigger part of small granules which have been sorted away into the pulp cords. The possibilities for larger grains to enter the pulp cords are, nevertheless, further increased. See diagram 4. More than half of them appear in the pulp cords at the diameter of 5 μ and 50 % at 6 μ. When the skew

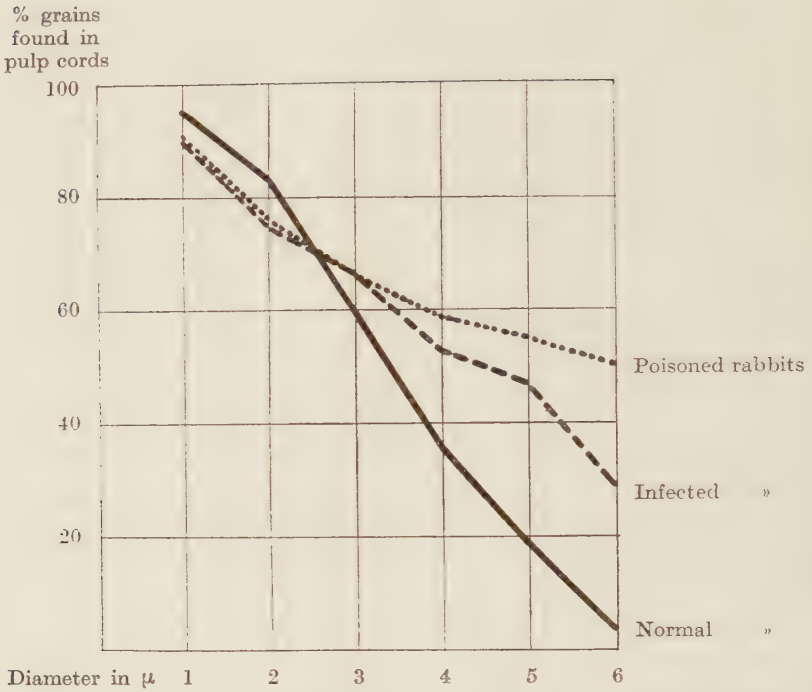

Diagram 4. Percentage of grains found in pulp cords at varying diameters in different groups.

distribution of the pulp material is subtracted from the skew distribution of the whole material, then there remains a normally distributed rest — the sinus-grains. They reveal a skewness reaching -0.40 ± 1.16.

Summing up the results from these two experimental groups, one can say that the difference between sinus- and pulp-grains is lesser when compared with the values found in normal rabbits. Moreover, the distribution of pulp-grains presents a skewness which to a greater extent agrees with that of the whole material. This ends in a normally distributed sinus-content of the granules.

From these results we may conclude that the sinus wall under these experimental conditions still shows a filtering function, viz. that of separating the injected particles according to their size. The filter is, however, more leaky and allows a greater part of larger particles to enter the pulp partitions than in normal rabbits. As a quite natural outcome of this is the fact that the distribution of the

pulp-grains is quite similar to that of the material at the outset. On the other hand, the differences between the means for the sinus and pulp fractions show that filtration still proceeds from the sinuses into the pulp cords. The circulation type is, in spite of the acute splenic swelling, therefore divided, as in the normal rabbits. HUECK's suggestion, as referred to above, that circulation in the dilated spleen should be open, can, consequently, not be proved.

Comparing the results in these last two groups with those obtained in normal rabbits, one will se some noticeable differences.

For instance, the size of pulp-grains is, as seen above, greater than in normal rabbits. The difference between the mean size of the pulp-grains in normal and infected rabbits rises to $0.51 \pm 0.03\ \mu$; the difference between the normal and poisoned ones to $0.74 \pm 0.04\ \mu$. Notwithstanding the skew distribution found in some of these groups, the difference is statistically significant. As the size of the pulp-grains must be influenced by the size of the stomata through which they have filtered, one may conclude that these pores in the last two experimental groups are larger, i. e. the filter is coarser. On the other hand, the values for the granules retained by the sinuses, show very small differences in all three groups. They are:

Normal rabbits: $3.52 \pm 0.02\ \mu$
Infected rabbits: $3.60 \pm 0.03\ \mu$
Poisoned rabbits: $3.66 \pm 0.03\ \mu$

These figures must be affected by the width of the afferent capillary, which, according to these results, does not seem to undergo any remarkable changes.

The histologic examination brings out also a different picture in these rabbits where an acute splenic swelling was produced. Despite injected gelatin, the flooding of the pulp cords with red cells is here a more frequent finding than in normal rabbits. This is the case, above all, in the group of poisoned animals, which also have the biggest spleens as well as the widest sinuses. From the histologic point of view it is evident that an increased amount of blood must be held accountable for the acute splenic swelling. Another difference here is the very infrequent occurrence of diapedesis of white cells — probably lymphocytes — through the sinus wall as compared with normal animals where it is a rather common view. See fig. 4. In the infected group there appear a few in every section, in the poisoned

one none at all. In the normal rabbits one discovers, moreover, at intervals capillaries opening into sinuses. See fig. 7. In the groups exposing an acute splenic swelling I have never found any such communications. In these spleens, however, the abundant red cells veil various parts of the finer structures and because of that one should not conclude that such connections do not exist.

The question now rises whether the crowding of the pulp cords with blood corpuscles in these groups is a vital phenomenon or if it appears *in agone* despite gelatin injection. With argumentation used in discussing the gelatin experiments, it might be repeated that this kind of injection seems to fix the red cells within their vital pathways. That the stomata in the last experimental groups should be so widened as to permit even great aggregations of red cells to enter the pulp cords, is not probable if one surveys the results of the rice starch test. The animal got a gelatin injection immediately before it was killed. Therefore it seems very likely that the extravasation of the red cells from the sinuses takes place during life in acute splenic swelling.

It is, nevertheless, possible that the filtration of only plasma through the sinus wall may be preserved by the occurrence of other phenomena. In most infectious diseases there ensues an increased formation of rouleaux, and the sedimentation rate is exaggerated. This is, as shown by FÅHRÆUS, a vital phenomenon. This increased aggregation of red cells may, as was the case in the gelatin experiments, protect the pulp cords from flooding with erythrocytes and may, thus, secure a separation of blood fluid from blood corpuscles. In my animal experiments there was not recorded any increment of the sedimentation rate until the pre-mortal gelatin injection was made.

What causes the acute splenic swelling? Trying to make a contribution toward the solution of this problem, I made one experiment. Two rabbits, which had 24 hours earlier been injected with the usual dose of phenylhydrazin hydrochloride, were exsanguinated and their blood was transfused into two other rabbits. Heparin was used as an anticoagulant. Applying FOLINS phenol test, I examined the plasma of the blood of "the donors" before transfusion as to the presence of phenylhydrazin hydrochloride in it. The transfused rabbits were tested after 24 hours in the usual way.

The found values were:

Sinus-grains: $M = 3.49 \pm 0.10\,\mu$, $n = 89$, $\sigma = 0.92 \pm 0.07$.
Pulp-grains: $M = 2.97 \pm 0.08\,\mu$, $n = 48$, $\sigma = 0.83 \pm 0.05$.

These figures reveal a greater resemblance with those found in the last experimental groups than with those in normal rabbits. The histologic examination showed, too, the same picture as in the acute splenic swelling, viz. flooded pulp cords and wide sinuses. The mean for the sinus width was: $32.35 \pm 1.05\ \mu$. Since there was no phenylhydrazin hydrochloride in the plasma, one can infer that the splenic change may be due to some erythrocyte properties, perhaps their abnormal shape.

Normal rabbits injected with epinephrin before being killed showed strongly contracted, almost exsanguinated spleens. Sections from such spleens were not usable for the measuring of test-particles because most sinuses are compressed, showing practically no lumina. A rabbit poisoned with phenylhydrazin hydrochloride and then injected with epinephrin intravenously instantly prior to killing, presented an altogether different histologic picture of the spleen. The distribution of the blood was namely the same as in other poisoned rabbits, and the means found for the size of the grains showed agreement with the values found in those others.

They were:

$$\text{Pulp-grains: } M = 3.16 \pm 0.11,\ n = 55,\ \sigma = 0.78 \pm 0.07.$$
$$\text{Sinus-grains: } M = 3.63 \pm 0.16,\ n = 35,\ \sigma = 0.95 \pm 0.11.$$

From this single experiment it might perhaps be suggested that the effect of epinephrin is altered in the rabbit spleen poisoned with phenylhydrazin hydrochloride.

As shown above, the spleens of the acute splenic swelling group were enlarged, and this seemed to depend on the increase of blood in them. A statistical significant increment in the sinus width can only be demonstrated in the poisoned group, in the infected group it is of the same order as in the normal rabbits. The abundance of erythrocytes found in the pulp partitions may perhaps, to some degree, form an obstacle for an overdistension of the sinuses. There must come to an equilibrium between the number of red cells in the sinuses and pulp cords.

Summarizing the results of animal experiments, one may conclude that a filtering function of the sinus wall has been realized. The direction of the flow runs from sinuses into pulp cords. The circulation is of the type which earlier has been called the divided. The sinus wall is, seen as a filter, more leaky in conditions producing acute splenic swelling. This effect seems to be brought about by widening of the stomata.

IV. The Human Spleen

Analysis of Alterations in the Sinus Wall Structure taking Place during Variations of the Sinusoidal Volume

The changes in the filtering effect of the sinus wall, as found in my animal experiments, have already been pointed out in connection with the discussion of the acute splenic swelling. These results make a more penetrating analysis of the wall's permeability at varying sinusoidal volumes desirable. Thus I will now proceed the investigation with the purpose of trying to elucidate the alternations taking place in the sinus wall at changings in the sinusoidal volume. This was brought about by studying a human autopsic material. It may appear as an adventurous enterprise to set out examining a functional phenomenon on material already dead and fixed. But, fortunately, there are in the sinus wall structure certain details which facilitate, not to say, directly encourage, such an analysis. The things, here suggested, are the annular fibrils running in circles around the sinuses. They constitute, in a manner, natural indicators for the state of contraction or dilatation in the sinus wall. By measuring the intervals between adjacent annular fibrils within sinuses of different width, one may arrive at an understanding of the changes that occur when the volume of the sinuses gets augmented, i.e. that part of the cyclic sinus activity which KNISELY calls "filling-filtering phase".

Measurement of the Mutual Intervals between the Annular Fibrils in Human Spleens

A necessary condition for making a reliable measurement of these intervals, is that one is able to stain reticulum in such a way that it gets clearly visible in the sections. Most adequate for this purpose are, no doubt, silver-impregnation methods, most of which, however,

are rather difficult to use. But a very handy and quite trustworthy modification of BIELSCHOWSKY's method, as published by SNOOK in 1944, brought on a great alleviation for the investigation. As the author has carried out most of these stainings in accord with this method, it seems well grounded to relate the main features of the staining process and experiences gained during its course.

Methyl alcohol has been used a good fixation aid. Any remarkable shrinkage I have not been able to observe. As a matter of fact, the shrinkage for spleen material equals almost zero in 48 hours. The pieces excised for histologic examination must be relatively large and thoroughly fixed before blocks are cut away from their centers for sectioning purpose. A piece of 3—4 cm in thickness is thoroughly fixed after 48 hours in methyl alcohol. In this way even sections from the soft spleens in acute septic splenitis, turn out well since one may then avoid malformations which, otherwise, are easily brought about by pressure from knife or against the bottom of the fixing vessel. Usual embedding in paraffin has been used. Sectioning in 20 μ thick sections. In the first part of this investigation concerning the fibrillar intervals the usual section thickness of 5—7 μ was applied. By using thicker sections, however, one gains a surveying power without losing the transparency or the possibilities of photographing even at high power magnifications.

Staining with silver ammonium hydroxide, according to SNOOK:

1. Deparaffinated sections mounted on slides are reinforced with a thin celloidin film. As soon as this has dried, the section is poured over with a solution 1 % of $KMnO_4$ slightly acidified with sulfuric acid. This pouring shall last for 1—5 minutes. Reticulum which is difficult to impregnate, for instance, in septic splenitis, requires, according to my experience, a longer treatment time than that stated by SNOOK, i.e. 1 minute. Rinse.

2. Bleaching in oxalic acid at 5 % until every trace of brown color has disappeared. This usually lasts half of a minute. Rinse.

3. Mordant through uranium nitrate at 1 % 2—5 seconds. Rinse.

4. Impregnation 1 minute in a bath containing silver ammonium hydroxide (according to KUBIE and DAVIDSSON). Rinse quickly.

5. Reduction in formol at 1 %. This is the crux of the method. When reducing during the time of 1 minute, as given by SNOOK, most human spleens get overstained, especially such crowded with red cells. The reason why is probably that precipitations occur, above all, in the erythrocytes. So, in my judgement, it is more safe to keep to the change of color during reduction. As soon as there appears a yellow-brown color, the staining is finished; but if the color begins to darken, there turns up at once the risk of overstaining. The reduction is complete in most cases already after a few seconds. This

leads one to recommend watching the reaction of the section through microscope. — Rinse.

6. Treatment with a solution of 0.2 % gold chloride during 1 minut. Rinse.

7. Fixing in a solution of 5 % sodium thiosulfate during ca. $^1/_2$ minute. After-staining with picro-fuchsin or other dyes.

This method gives, in general, very good results. Most difficult to stain are, according to my experience, the spleens flooded by blood. In spite of repeated attempts I remained unable in staining the rabbit spleens by this method unobjectionably. A specimen is seen in fig. 8. The cause of my failure must lie in the avidity of the red cells to absorb the silver. It seems as tho the material fixed in methyl alcohol would deliver nicer impregnated sections than those fixed in formol.

The well stained section offers a quite fascinating view. The spleen sinuses run crisscross in all directions. If their course is changing then their wall structure is so much more regular. See fig. 9. When the sinus long axis runs along the section plane the wall will appear as in the form of a lattice the bars of which represent the annular fibrils. If the sinus is cut at right angle to its axis, its lumen appears as a circular hole. Wherever the annular fibrils are cut in sectioning, there one sees the wall as a dotted line. Here and there one discovers a sheathed capillary opening into a sinus. See fig. 14. Such connections are more easily found in the thicker sections. While searching for such communications, we notice how the field-view behaves like a fixation picture. Having watched a sinus for a while, one suddenly recognizes an afferent capillary.

All spleens — normal as well as amyloid, leukemic ones and such in Hodgkins disease — here examined and stained with Snook's method, show a clearly outstanding sinusoidal structure. Tho strange enough, yet this type of structure is kept intact even in septic splenitis in spite of the almost liquefied form. That the sinuses are not seen in some sections, can be ascribed to technical failures. At a new attempt, however, one will often achieve a better result. If Knisely maintains that the sinuses are the functional unit of the spleen, then it is evident from these sections, stained according to Snook, that they, at least in man, also constitute its anatomically largest part.

The boundary-line between the sinuses and the collecting veins is not very strictly defined. Only when the veins are enclosed in

Table showing the mean values of the intervals between the annular fibrils in various diseases.

Width of sinuses in micra		Intervals in micra						
		10 μ	20 μ	30 μ	40 μ	50 μ	60 μ	80 μ
Chronic congestive heart failure	M ± ε(M)	3.30 ± 0.07	3.46 ± 0.05	3.57 ± 0.06	3.88 ± 0.13	4.36 ± 0.21	4.93 ± 0.19	4.92 ± 0.28
	n	170	499	423	111	72	57	36
	σ	0.88 ± 0.05	1.02 ± 0.03	1.19 ± 0.04	1.39 ± 0.09	1.75 ± 0.15	1.44 ± 0.13	1.68 ± 0.20
septic splenitis	M ± ε(M)	2.95 ± 0.21	3.16 ± 0.05	3.06 ± 0.05	3.39 ± 0.07	3.40 ± 0.12	3.44 ± 0.14	4.36 ± 0.27
	n	21	597	446	332	90	74	36
	σ	0.95 ± 0.15	1.12 ± 0.03	1.14 ± 0.04	1.19 ± 0.05	1.10 ± 0.08	1.24 ± 0.10	1.59 ± 0.19
normal cases	M ± ε(M)	2.18 ± 0.11	2.41 ± 0.04	2.40 ± 0.05	2.28 ± 0.07			
	n	45	211	181	104			
	σ	0.73 ± 0.08	0.63 ± 0.03	0.71 ± 0.04	0.74 ± 0.05			
47[1] mixed cases	M ± ε(M)	2.93 ± 0.06	3.17 ± 0.03	3.19 ± 0.03	3.28 ± 0.05	3.75 ± 0.10	3.88 ± 0.11	4.64 ± 0.19
	n	280	1679	1446	742	207	201	72
	σ	0.97 ± 0.04	1.06 ± 0.02	1.17 ± 0.02	1.24 ± 0.03	1.43 ± 0.07	1.62 ± 0.08	1.61 ± 0.13

[1] The mixed group consists of the three groups above and to these values from spleen sections from various other disorders, such as liver cirrhosis, trombocytopenic purpura, anemias and septic endocarditis.

trabecular sheaths, we can, no doubt, recognize their true nature. The intralobular veins have namely practically the same structure of their wall as spleen sinuses, tho their annular fibrils are possibly somewhat more scattered.

The distances between adjacent annular fibrils have been measured in sinuses of varying widths in at all 47 spleens from cases succumbed from different diseases. Most material is derived from pathological cases, only few originate from peoples who have died a violent death and may be stated as normal. Where the sinus wall gave the view of a lattice, I have measured the interval between the middle points of adjacent fibrils, and where it appeared as a dotted line, the intervals between the dots. For these measurements I have not used such sinuses as have an axis sloping toward the section plane where one, consequently, had, as it were, to climb from bar to bar with the aid of the micrometer screw. The values found for different sinus widths have been gathered and their means calculated. For results look at the table on the other page.

These results make it clear that sinuses with a wideness ranging from 10 to 40 μ have, approximately, the same intervals between their annular fibrils. From the sinus diameter of 50 μ and more there appears, however, a statistically significant increase in the distances.

The rodshaped cells, as being the second element in the sinus wall, are harder to analyze because they are more difficult to stain. In general, the staining is best done with Heidenhein's iron hematoxylin, which, nevertheless, cannot be combined with the method of SNOOK. Yet this method when united with "Wasserblau-Orcein-Safranin", may give a satisfactory staining. Even VAN GIESON stains them sometimes tho with varying intensity. Some structures in the rod-cells absorb silver. See fig. 11. This observation is already made by FOOT in 1927.

Even within the rod-cells there is a structural detail that may serve as an indicator of their eventual elasticity. This detail consists of a striation appearing at regular distances which runs all along the cellular length. Stained with iron hematoxylin, it is seen as darker sections or fragments. The distances between two such adjacent sections rises to ca. 2 μ. One can localize these fragments at the basal part of the cell, which turns away from the sinus lumen. In material impregnated with silver one can at times trace these sections. They are always, then, situated in those places of the rod-cell which lie between

two adjacent annular fibrils. This fact fortifies the strong impression of a peculiar regularity that overwhelms one engaged in the study of the human spleen sinus wall. Another illustration of this, is the fact that the darker sections in all rod-cells belonging to the same sinus, are located at the same hight altho their nuclei, as shown by JOLLY, lie on different levels. See fig. 11, 12 and 13.

When analyzing the structural changes in the sinus wall at different sinus widths, one may judge from these darker sections whether the rod-cells get lengthened or widened during sinus dilatation. As there occur no noticeable alterations either in their relative distances or in their breadth, we may conclude that such do not take place. Perhaps one may, however, admit that in wider sinuses they are somewhat broader. See fig. 13.

In order that these measurements should constitute a basis of preceding analysis, it is needful that the assessed widths of the sinuses really represent sinuses of different dilatation degree, except the exceedingly wide ones, which probably consist of collecting veins. That spleen sinuses have power to alter their volume is widely accepted. This also I could show in my animal experiments, for instance, by comparing the widths of the sinuses both in normal rabbits and in those poisoned with phenylhydrazin hydrochloride. Another evidence of this is the decreasing sinus diameter after injection of epinephrin. The sections stained, according to SNOOK, give one more reason supporting the ability of the sinuses to alter their volume, i. e. the fact that the annular fibrils are finer in wider sinuses and vice versa. This is presumably due to the stretching of the fibrils. He who accepts KNISELY's conclusions, the truth of which my animal experiments are likely to prove, can in these sinuses of different widths see various degrees of filling states. The finding of the regular intervals between the annular fibrils, despite increasing sinus diameters, points to the fact that the sinuses while increasing their volume, do not lengthen but only dilate. The increment in the periphery of the annular fibrils, i. e. their elasticity, constitutes the basis of the enlargement of the sinus volume. If the sinus diameter widens from 10 to 40 μ, this implies a lengthening of the periphery from 32 to 120 μ. As the rod-cell do not broaden in comparison with that, the stomata will, consequently, grow wider.

If one designates the distances between the annular fibrils, always remaining the same, as the *hight of the stoma*, then it is evident that

it is its *breadth* which is variable. That the rod-cell is about 2 μ broad, as WEIDENREICH says, seems to be true. A sinus with a diameter rising to 10 μ and, consequently, a periphery up to 32 μ, is, then, only capable of holding 16 rod-cells in cross-section. When this very sinus reaches the width of 40 μ and the peripheral length of 125 μ, the breadth of the stomata have increased proportionally to this, according to this schedule.

Diameter of sinus	Periphery of sinus	Breadth of stomata
10 μ	32 μ	0 μ
20 »	64 »	2.0 »
30 »	94 »	3.9 »
40 »	125 »	5.8 »

The breadth of the stomata is, thus, correlated to the size of the sinus. As the stoma-hight, however, does not undergo any changes there must come about a stage at which the hole appears as a square, i. e. at the periphery of 86 μ corresponding to the sinus-diameter of 27 μ. The exceeding of this diameter does not imply any increased permeability for unelastic spheric particles inasmuch as the hight of the stoma actually stops their transfer into the pulp cords. The widening of the stoma breadth allows, nevertheless, a letting out of erythrocytes into the pulp partitions when a certain degree of sinus dilatation is reached. If calculating with pure figures, one will find that the stoma breadth reaches the value of the red cell diameter at a sinus width rising to 49 μ. A leaking out of red corpuscles may, however, take place even earlier if we consider the elasticity of the erythrocyte. It should, therefore, be stressed that the reaction KNISELY observed in the dying spleen might have been a dilatation of the sinuses, unnoticeable because of the slight increment of their diameter. It should, furthermore, be emphasized that the increase of the permeability of the sinus wall probably is brought about through an alteration of the shape of its stomata. They turn from squares into rectangular slits.

This mechanism with a permeability increasing as the widths of the sinuses broaden, elucidates the results obtained from my animal experiments. The group of them showing an experimentally produced splenic swelling had a greater mean for the size of the pulp-grains indicating a coarser sinus wall filter. But the wall found in the rabbit we cannot in detail compare with that found in man since it has the

character more of a network of circular meshes than that of a lattice. In principle, however, the changes may be the same. It might, thus, not be altogether unjustifiable to state that the degree of acute splenic swelling is also a measure of the stoma breadth in the spleen sinus wall.

The rod-cells are considered to be elastic, even contractile, altho the reasons for it appear rather weak, especially when more closely scrutinized. Weidenreich and von Herrath, already presented, espoused the contractility of these cells because, in their opinion, the human spleen contains too little of smooth trabecular and capsular muscles in order to pour out the blood from it by their contraction. But Foot is, on the other hand, inclined to accept the contractililty of the rod-cells as due to their striation. My analysis of this matter points, however, to the fact, as already stated, that the intervals between the annular fibrils are rather invariable and that the darker sections in the rod-cells do not undergo any considerable changes. This should indicate the rod-cells are not contractile.

However, one must always remember I made my analysis on dead material. The intervals found are certainly not representative of the measures in the living spleen since the setting-in of death involves a relaxation of *tonus* and *turgor*. During life the fibrillar intervals may be either longer or shorter and the rod-cells contracted but relax at the point of death. It seems, nevertheless, acceptable that this relaxation occurring *in agone* is uniform, and this is why one may consider the post-mortem finding reproduces only the living conditions on another scale.

The Sinus-Sphincter

It is, indeed, not easy to decide which lumina pertain to spleen sinuses and which to collecting veins. The trabecular veins are, however, easily distinguished. Between them and the spleen sinuses there exists a system of collecting veins having walls of the same structure as the sinuses. They may, notwithstanding, in general be wider than the sinuses and their layer of rod-cells is closer than in a sinus of the same diameter. While measuring the distances between the annular fibrils, one finds that sinuses rising above 50 μ in breadth have more scattered fibrils. This may be explicated in the fashion that they are, as a matter of fact, veins. Even Foot holds, likewise, the opinion that the intralobular veins are built up as spleen sinuses.

If so, their fenestrated wall should have the same property as the sinus wall, i. e. that of being permeable for plasma and smaller particles. It seems very likely that the blood fluid having filtered out from the sinuses, returns to the circulatory pathway through the perforated walls of the collecting veins. While traveling through the spleen, plasma passes, accordingly, three filters. First, out from the sinuses through the sinus wall; next, through the cellular masses of the pulp *reticulo-endothelium*, and finally, through the venous wall into the veins. This scrupulous filtration is most likely of biological and serological importance.

Due to the similar structure of spleen sinuses and collecting veins the boundary-line between them must be of functional significance, provided the sinus is the working unit. This stands also clear from KNISELY's review of the sinus activity. Because of an observed contraction at the venous end of the sinus starting the filling phase he assumed the existence of a sphincter at this place. Having made some observations in the human material, I will presently give an account of the eventual morphological basis of the sphincter activity.

In the spleen sinuses the rod-cells run longitudinally crossing the annular fibrils at right angles. In the veins the appearance of the wall is the same, viz. the rod-cells, likewise, cross their annular fibrils at right angles. The same rod-cells as constitute the spleen sinus wall, continue their course in the venous wall. At the venous outlet from the sinuses they, however, change their way. They are no longer running at right angles towards the annular fibrils but crossing them at acute angles. A rod-cell that in the sinus has run along one wall may at the outlet cross over to the opposite side. Thus, there is formed a more or less spiral-shaped screw of rod-cells at the sinus outlet. Such deviations in the running course of the rod-cells are, certainly, seldom seen in the same section, but, nevertheless, often enough as to be, beyond question, real phenomena. See fig. 15, 16 and 17.

In his work of 1927 FOOT remarks that the sinusal endothelium shows at the sinus outlet a more complicated run and, to some extent, radiating course. To the best of my knowledge I have not found similar observations stated anywhere in the literature bearing on this subject. The significance of this altered running-course is rather problematic. It is, however, possible that the rod-cells by their spiral-form arrangement may constitute the basis of a mechanism functioning as a sphincter. Whether the rod-cells are elastic ore not, it is obvious

that these parts of them crossing over to the opposite wall side must be subjected to a higher degree of stretching during sinus dilatation than those parts which do not cross over. This cellular extension may interfere with the tendency of the annular fibrils to dilate, and so the rings localized at the sinus outlet get incapable of dilating. They may even get narrower. The effect is that the sinus outlet constricts.

This peculiar endothelial sphincter is the only structure found at the sinus orifice which may constitute a morphological basis of the activity watched by KNISELY as starting the filling phase.

Structural Modifications of the Sinus Filter under Certain Conditions

An analysis of the porosity of the sinus filter I made on material, mostly gathered from pathological cases. The main part consists of spleens manifesting either acute splenic swelling or chronic cardiac congestion. However, I have also examined some normal spleens, derived from peoples who have accidentally succumbed to violent death. In these spleens the findings were the same as in others, i. e. the intervals between the annular fibrils are invariably the same even tho the sinus widths may vary. But these distances are, nevertheless, shorter in normal cases than in pathological ones. Thus we may unite the values obtained from different sinus widths into a common mean representing the mean distance for each group. These means are:

Normal cases:..... 2,42 ± 0,03 μ
Chronic cardiac congestion:..... 3,57 ± 0,03 μ
Acute splenic swelling:..... 3,31 ± 0,03 μ

The difference between the normal cases and the pathological cases is significant and obviously great.

To the group of cardiac congestion I have referred the spleens derived from deaths of congestive heart failure; to the group of acute splenic swelling spleens, showing at autopsy a soft pulp, and to the normal group, spleens from normal cases. The values are found in the table on page 65.

Even in other respects one can point out differences between normal and certain other cases. The structure of the spleen sinus wall does, as a matter of fact, show during certain conditions a modification of the very wall anatomy, viz. of the annular fibrils. This will now come up for discussion.

Even at an early stage of the investigation, it became evident that the annular fibrils were difficult to detect in spleens of newborns, but not so in case of grown-ups. Here I found them running principally parallel to one another with few anastomoses. The rod-cells crossing the annular fibrils complete the grid of rectangular pores. In the fetal spleen there are hardly any annular fibrils. Here we find the sinus wall as built up by a layer of reticular filaments forming a network of ring-shaped meshes, approximately the same in wideness. It suggests the idea and sight of a wire-netting, altogether different from the lattice in adult spleens. A similar network is found in the rabbit spleen. See fig. 20, 21 and 8.

It is conceivable that this structure in the fetus transforms into the annular fibril one of the adult through reduction of its longitudinal reticular elements. The most pronounced network, such as suggested above, is found in the youngest fetuses. Childs at the age of one year reveal, to a greater extent, the form of rectangular lattice of grown-ups. I have examined 15 child spleens. Except one derived from a eight months old boy with a severe burn, all of them showed the netting-structure, most striking in the youngest ones. Here I could not with any certainty measure the intervals between the annular fibrils.

Any noticeable change of the netting-structure in different pathological conditions I was not able to record. This was true of all spleens from newborns, no matter, whether they died from fetal asphyxia, capillary bronchitis or other infectious diseases.

This structural peculiarity in the sinus wall of the fetal spleen was, as already stated, observed by Billroth in 1860. Here he found the difference between the intervascular netlike tissue, i. e. the pulp cords, and the capillary veins, i. e. the spleen sinuses, to be smaller and more difficult to establish than in the adult spleen. The whole red pulp appears, according to him, in the shape of a uniform network, where one could discover the sinuses only after injection of the vessels. This queer framework of the child spleen sinus wall, early discovered by Billroth, has since his time, as far as known, not received any attention. For verification of facts see fig. 20 and 21.

Concerning the material gathered from adults I found this network also in another group, viz. that of the chronic leukemias, including both the myeloid and the lymphatic type. Here the sinus wall is almost an exact copy of that in case of newborns. I have examined the spleen in 9 leukemias, 5 chronic and 4 acute ones and found the netting structure of the sinus wall strongly developed in all chronic cases but at most only indicated in two of the acute ones. The change appears so radical that one can no longer make any measurements with any assurance of accuracy. See fig. 21, 23, 26 and 27.

Even in liver cirrhosis with splenomegaly there occurs an alteration in the structure of the sinus wall. It is, however, of another type than that in the foregoing groups. Tho I have examined only two cases, yet both of them showed the same change, viz. that consisting of great many longitudinal anastomoses between the annular fibrils. See fig. 24. These filaments are thus preserved, and measurements may be performed. This change I also found in cases of chronic cardiac congestion, tho not so pronounced here where the anastomoses were more scattered. See fig. 25.

The suspicion may rise that these additional filaments — here interpreted as anastomoses — should really be parts of the reticular fibers derived from the pulp cords, and get pressed against the annular fibrils during sinus dilation. But this is, certainly, not the case, because upon a closer examination one will find that they are real anastomoses connecting only two adjacent annular fibrils with each other.

The changes taking place in leukemia, liver cirrhosis and cardiac congestion were, as already noted, discussed by Matsui in 1915; but since then no one has payed any attention to them. My investigation on material, stained according to Snook, confirms, however, his findings.

The most remarkable macroscopic change appearing in septic splenitis in man, is an alteration in the consistency of the spleen. The whole organ grows softer and its pulp is liquefied. This transformation makes, as previously stated, a histologic analysis difficult. It has been suggested that the change is due to a postmortal autolysis of the spleen taking place very rapidly.

If applying such precautions as outlined in previous chapters while collecting the material, one will then be able to present sections,

stained according to Snook, with a well preserved structure even tho the organ reveals obvious signs of soft splenic swelling. The sinuses appear mostly very dilated; the annular fibrils are exceedingly thin and seem to be vigorously stretched, besides being difficult to impregnate. There is no increase in the number of anastomoses. Some sinuses are crowded with red cells (see fig. 19), others are empty. More often here than in sections from other cases one finds the splenic structure destroyed. This is, in my opinion, due to the fact that these spleens are so susceptible to manipulation. Any variety in the values for the intervals between annular fibrils at different sinus widths is still not found.

The acute form of myeloid leukemia showed in four examined cases a sinus wall structure as in every respect agreeing with that found in septic splenitis, to which even the macroscopic picture corresponds. The rod-cells within preserved parts of the spleen are, even in cases of acute splenic tumor, in contact with the annular fibrils, and seem to be unaltered.

Any other conditions showing positive changes in the sinus wall structure have not so far been detected. *Purpura trombo-cytopenica* revealed in three cases, successfully impregnated, a sinus wall structure agreeing with the normal splenic framework.

Interpretation of the Changes Taking Place in the Reticular Layer of the Sinus Wall

We have already found that the sinus filter porosity increases as the sinus volume expands. This I have explained previously in such a way that the stomata widen as the sinus periphery lengthens. These findings one ought to bear in mind when interpreting the results arrived at while studying the pathological material. This was drawn from splenomegalies, partly acute, i. e. the septic splenitis, partly chronic, i. e. leukemias, liver cirrhosis and chronic cardiac congestion.

In acute splenic swelling in man one finds evidence for a dilatation of the sinuses. This accords with the findings in my animal experiments. There are, consequently, good reasons for concluding that the stoma breadth enlarges in these cases, and the sinus filter turns more leaky. This fact may in all likelihood be responsible — at least to some part — for the finding of the soft consistency of the spleen

in acute splenic swelling in man. The red pulp, i. e. the main bulk of the human spleen, is built up of countless vessels separated by cellular pulp cords. The boundary-lines between pulp partitions and spleen sinuses, on one hand, and between pulp cords and collecting veins, on the other, are formed by porous walls, which — at least in case of the sinus wall — have been showed to act as filters. Now, if they turn more leaky, which we have good reason to consider as true in the case of sinus dilatation and, by way of analogy, quite likely also in that of the venous wall when the vein distends, then there will, consequently, rise a wider communication between sinuses, pulp cords and veins. Then, the contents of these vessels will easily pass from sinuses to pulp partitions, for instance, or in the opposite direction, according to existing pressure differences. The conditions here are comparable with those arising during perfusion after the manner of WORONIN. Several histologists used the fact that while perfusing the spleen one washes away the cells of the pulp cords as a proof of an open circulation. It remains, however, clear that an increased filtration from the sinuses into the veins by way of the pulp cords can also bring pulp cells along with it, as really also happens during perfusion.

A wider communication between sinuses and pulp cords, and possibly also between veins and pulp cords, explains, in my opinion, the change in the consistency of the spleen as being the most characteristic find at autopsy in infectious diseases. The cellular content and the blood fluid are no longer retained within walls having continuous endothelial layers. Thus it happens that at manipulation everything seems to be liquefied.

This kind of argumentation leads up to the assumption that the change of consistency as observed in the dead body, is really caused by a widening of the sinus wall stomata during life. BARCROFT found the dead spleen smaller than the living one. This alteration in volume is probably brought about by voiding the sinuses, thus through their contraction. But any such a one has apparently not occurred in spleens showing acute splenic swelling since the sinuses there are wide and partly blood-filled, even *post mortem*. This finding we can compare with the deficient reaction to epinephrin of the spleen of the rabbit poisoned with phenylhydrazin hydrochloride.

The intensified communication between spleen sinuses and pulp cords should result in a lessening degree of separation between the

blood fluid and the red corpuscles inasmuch as these cells are carried away together with plasma through the stomata. It is, however, possible that separation *in vivo* is maintained thanks to the increased rouleaux-formation taking place in infectious diseases. This may — as was the case in my gelatin experiments — make the red cells stay intravascularly.

With respect to the morphological reaction relative to infectious illnesses the child spleen presents a type different from that of the adult organ. Pulp softening does not occur. Neither does it appear in chronic leukemias nor in cases suffering from chronic cardiac congestion, which, nevertheless, are usually exposed to intervening infections. Nor could I observe any softening of the rabbit spleens inoculated with *streptococci* in the experimental groups.

The explanation of the fact that these spleens react in another way one must, in my opinion, seek to find in the structural differences of their sinus wall as compared with the normal human one. The netting-structure of meshes — as seen in fetuses, rabbits and chronic leukemias —and the multiplied longitudinal anastomoses, as found in liver cirrhosis and cardiac congestion, must really cause the broadened stomata to grow tighter in spite of dilating sinuses. Consequently, there does not follow an increased communication between sinuses and pulp cords to the same extent as in the normal spleen, previously considered. This is best illustrated by comparing the acute and chronic leukemias both in regard to their gross-appearance and the reticular structure of the sinus wall. In acute cases we find a soft consistency with annular fibrils kept intact, but in chronic cases a firm consistency and netting-structure of the sinus wall.

In this connection one should remember that it was in child spleens that Billroth made his discovery of the closed pathway; the sinus wall seems, consequently, to have a lesser degree of permeability, even for injection matters.

What can be the cause for this modification of the sinus wall structure? The netting-structure of the wall appears, as far as I know, in but two cases, viz. those of fetuses and chronic leukemias. Following this line of thought, one might search for the cause in the lining of the walls with myeloid elements. During fetal life the spleen is a myeloid organ and the same appears to be the case in chronic myeloid leukemias. But this explanation is not applicable to the other cases, viz. liver cirrhosis and cardiac congestion. In

these spleens we do, certainly, not come across any circular meshes in the sinus wall, but find, nevertheless, a tightening of it through longitudinal anastomoses between the annular fibrils. There is possibly another explanation at hand. The modification bears apparently the character of a reinforcement. Except in fetal cases, it appears in chronic enlarged spleens, which *in vivo* had probably widened sinuses. Concerning liver cirrhosis and chronic cardiac congestion there are reason to suggest an increased pressure within the portal system, i.e. a portal hypertension. This should interfere with the voiding of the sinuses. JÄGER brought about such a sinus dilatation in dogs spleens by producing in different ways (1927) a portal obstacle, either by tying *vena porta* or by making a reverse of ECK's fistula, i.e. *vena cava* was anastomosed into *vena porta*. Here the dilatation of the sinuses reached a high degree, especially in the last case.

May this factor — a portal hypertension — be the common explanation for the modified sinus wall structure? The fetal circulation does, through the shunting of *vena umbilicalis* to *vena porta*, offer a certain parallel to the reverse of ECK's fistula of JÄGER. Nine tenths of the umbilical venous blood travels, according to BARCLAY and coworkers (1944), through the liver. HASELHORST (1927) measured the pressure in the umbilical vein in three full term human fetuses and found, on an average, the considerable value of 26 mm Hg. I have not been able to secure any data about the portal pressure in adults. In 1909 BURTON-OPITZ, however, measured it on dogs and found the value of 9 mm Hg. BARCLAY and associates could during their investigation register a splenic contraction in the lambkin at birth. This may, however, have many other causes and does not necessarily point to an enlargement of the spleen during fetal life. The portal pressure in leukemias is not known. Myeloid infiltrations in the liver as well as such in the spleen as influence the intralienic veins, may possibly produce a venous congestion making it more difficult for the sinuses to empty their contents. An analogous explanation may be applicable to the digestive splenomegaly, as observed by KNISELY, among others. In 1934, HERRICK, MANN, ESSEX and BALDES were able to record a great increase, rising to 74 per cent, in the flow of blood through the liver of a dog after a meal of meat and cereal. This may account for a temporary

rise in the portal pressure resulting in a multiplication of the number of storing sinuses.

Summarily: a study of the pathology of the spleen sinus wall unveils that certain alterations occur in its reticular layer. These changes seem to bring on a tightening of the sinus filter, suggesting that the preservation of its filtering ability should in some way or other be significant. The function which is thus maintained, is the separation between red cells and plasma. The storing of erythrocytes occurring during this separation is evidently the same phenomenon which has been interpreted as the splenic reservoir function for red corpuscles. This function brings, however, about qualitative changes in the red cells which, to some extent, diminish their value as an emergency reserve. Because of this fact it seems probable that the reservoir function, as observed by BARCROFT and BINET, is only the other side of the shield and that the essential function is the deplasmatization of the red cells. Its physiologic significance has been elucidated by the works of BERGENHEM and FÅHRÆUS. It leads to a destruction of red corpuscles.

V. Summary and Conclusions

The circulatory pattern of the red splenic pulp is, in spite of numerous investigations facing this problem, still not elucidated. Reviewing its literature, one finds three different theories.

1. The theory of closed circulation. It holds that the blood in the spleen should everywhere, as in other organs, follow endothelially lined pathways. This scheme of thought is, however, nowadays abandoned, because several histologists have convincingly proved the nature of the sinus wall as being that of a fenestrated membrane, i.e. perforated by numerous slits — stomata — through which the sinuses communicate with the surrounding pulp cords.

2. The theory of open circulation. Its adherents claim that in the spleen such exceptional conditions prevail that the arteries empty into the tissue spaces, or — in other words — that the sheathed capillaries should pour their blood into the pulp cords from which it would pass through the sinus wall stomata in order to enter the veins. That the pulp cords in histologic sections appear invariably crowded with red cells, this fact is still a strong evidence of the validity of this theory, as it seems irresistable. It is also the one most accepted and referred to in text- and handbooks.

3. The theory of divided circulation. The American anatomist Knisely (1936) studied circulation through living spleens in experimental animals by means of a special method — transillumination. He found the splenic ciruclation showing certain features altogether characteristic of this organ. Thus, whole blood enters the sinuses from the afferent capillary and then the red cells are continously stored in them during various lengths of time, rising to several hours. During sinus filling, plasma filters continously through the sinus wall into the pulp cords in order to pass into the veins. Knisely's circulation type — in this work designated as the divided circulation — has, however, not been widely accepted. Using his method, after-examiners have not been able to verify his results.

Altho his theory is referred to in present literature, most writers, however, do not agree with him, but take a stand for open circulation.

Present investigation has been carried out in order to elucidate the circulatory pattern of the splenic red pulp.

As seen from above, there are, from the standpoint of the various theories, three fundamentally different explanations available for the crowding of the pulp cords with red cells. For the theory of *open* circulation this phenomenon yields an essential evidence. According to the *closed* circulation theory the red cells are interpreted as due to extravasations artificially produced, i.e. simply as consequences of hemorraghes. According to the theory of *divided* circulation, finally, the pulp cords crowded with erythrocytes must, likewise, be looked upon as being an abnormal occurrence. KNISELY found the withdrawal of erythrocytes to be, in some unknown way, connected with the timing of very death.

Both the open and the divided theory, thus presupposes a transition of blood or parts of it through the lattice-like structure of the spleen sinus wall. The direction of this passage is, however, completely the reverse in both cases. The followers of the open circulation claim that whole blood should travel from pulp cords into sinuses; according to the divided circulation, plasma should filter from sinuses into pulp cords.

The conception of the lattice-like structure of the sinus wall fed the idea of studying what effect one might achieve by altering the dispersity of the blood suspension, with regard to the distribution of the red cells between the different structural parts of the spleen, viz. spleen sinuses and pulp cords. By injecting gelatin intravenously, one produces an increased aggregation of red corpuscles (FÅHRÆUS). In connection with such an injection in rabbits there was realized a change in the histologic appearance of their spleens, inasmuch as the crowding of the pulp cords with erythrocytes decreased. This was probably due to the fact that gelatin brought about aggregations of red corpuscles too large to pass through the stomata. Following the pathway of open circulation these aggregations should have brought about an increased pooling of blood in the pulp cords.

If one recognizes the peculiar character of the sinus wall, it seems conceivable that even a slight alteration in the shape of the red cells would produce a change in their filterability. It became also evident that substantially the same effect with respect to the splenic

structure was caused by injecting saponin which brings about a spherocytosis of the red cells, perhaps also a lessening of their elasticity. Thus it is obvious that the sinus wall constitutes a sort of mechanical filter having a special filtering effect which, as judged from the saponin experiments, directly corresponds to the size of the erythrocyte.

For the purpose of obtaining a closer characterization of the qualities of this sinus filter, I worked out following method. After an injection of a rice starch suspension containing grains of various size (mean diameter 4 micra), I have ascertained the locations and dimensions of these grains in different parts of the spleen after the killing of the animal. (In order to secure a greater surveyability in the histologic sections, these experiments were, as a rule, combined with the injection of gelatin or saponin immediately prior to the killing of the rabbit.) Upon injection of such a starch suspension, one finds a great number of grains in the pulp cords at microscopy. It is, consequently, substantiated that there must be a communication between the blood vessels and the pulp cords so large as to permit transition of starch granules. The existence of such a communication is certainly not compatible with the view of closed circulation which also, as stated, represents a deserted theory.

When comparing the size of the grains refound in sinuses, respectively pulp cords, one finds that the sinus-grains are larger. Their mean diameter rises to 3.51 $\pm$ 0,02 μ and that for the pulp-grains to 2.70 $\pm$ 0.01 μ. Even from these experiments it is obvious that the sinus wall acts as a filter which from a suspension, such as used here, retains larger particles, but allows smaller ones to pass.

The tightness of the sinus filter may, in a way, be characterized by the value above showing the mean diameter for the grain refound in the pulp cords. Another understanding of its filtering effect one might get through the experimental results establishing that 81 per cent of grains with a diameter of 5 micra still are kept inside the sinuses while only 5 per cent of those having a diameter of 1 μ have been retained.

From these results it is also obvious that filtration goes from sinuses into pulp cords. KNISELY'S interpretation of the sinus activity can be stated as verified through these experiments. The theory of open circulation is thus proved false.

It is, however, difficult to judge from these experiments whether

the crowding of pulp cords with erythrocytes is a phenomenon arisen *in agone* or not. But as KNISELY's observations on other essential points have through this investigation been indirectly verified, it seems that one is also justified in accepting his thesis that the extravasations of red cells should be an agonal occurrence. This assumption, then, involves, as a natural consequence, the statement that the physiologic function of the sinus filter is to separate red cells from plasma or, in other words, to deplasmatize the red cells.

A necessary condition for the storing of red cells in the sinuses is, then, that they can be shut off from the collecting veins. According to KNISELY, there is also a sphincter mechanism between the sinus and the vein belonging to it. It is thus of interest to state that there is possibly a morphological basis for such a sphincter. This basis consists of a deviation in the running course of the rod-cells. A hypothesis about the mode of action of this sphincter has been proposed.

Experiments carried out by FÅHRÆUS & BERGENHEM, MELLGREN and NORDLANDER illustrate the physiologic significance of the deplasmatization process. The first two of them have proved that red corpuscles devoid of their plasma at body-temperature *in vitro* show alterations indicating hemolysis, viz. spherocytosis as well as decreased rouleaux-formation and sedimentation rate. MELLGREN established in a convincing way that splenic reservoir blood in cats revealed changes of the same nature in every respect, and found also signs suggesting hemolysis. NORDLANDER's experiments made it clear that deplasmatized erythrocytes from horse, man and cat *in vitro* at body-temperature emitted potassium. This procedure could, likewise, be shown as taking place in the reservoir blood from cat.

For the purpose of investigating the ability of the sinus filter to alter its tightness, I tested, using the same method as before, its porosity in rabbits which had an acute splenic swelling, experimentally produced. It appeared that under such conditions the distribution of the starch grains was altered inasmuch as their mean size in the pulp cords was larger than that of normal rabbits. Grains with a diameter of 5 micra were up to 50 per cent situated in the pulp

cords. The sinus filter is, accordingly, more leaky in acute splenic swelling as compared with normal spleens. No doubt, this is the cause for the increased number of red cells that one finds in the pulp cords in such a condition.

In order to elucidate how these alterations in the tightness of the sinus filter are brought about I made an analysis of the organisation of the spleen sinus wall at different sinus widths. This I performed on a human autopsy material, stained according to SNOOK. The results then obtained revealed that the intervals between the annular fibrils — here called the hight of the stoma — are practically unchanged at various sinusal widths. At increasing sinus volume there appears, in point of principle, only a dilatation of the sinus without any lengthening of it. As the rod-cells do not broaden in any noticeable degree during dilatation an incerase of their intervals, i.e. a broadening of the stoma, will naturally follow. This increasing stoma breadth is probably the cause of the diminishing tightness of the sinus filter at sinus dilatation.

In acute splenic swelling in man one finds at autopsy a typical softening of the consistency of the spleen. This may, to some extent, be caused by an increased amount of blood in it but also, in my opinion, through the widened intercourse between sinuses and pulp cords, rising as a consequence of sinus dilatation. Plasma and corpuscular elements are, without meeting any resistance, transported from sinuses into pulp cords and *vice versa* through the broad stomata. At autopsy this appears as a dissolution of the constituents of the organ. The decrease in consistency might, in other words, correspond with a coarsening of the sinus filter.

There are difficulties in differentiating between collecting veins and sinuses. These veins have, as it seems, a wall structure of their own which greatly resembles that of the sinus wall altho the intervals between the venous annular fibrils might be greater. KNISELY was not able to find out the fate of the blood fluid which filtered through the sinus wall, but thought it probable that it joined the erythrocytes in the veins. The fact that the walls of the smaller veins are fenestrated makes it conceivable that plasma, having passed the pulp cords, is pushed back into circulation by a transition through the stomata of the venous walls, now in direction from pulp cords to blood vessels. This involves the suspicion that even the venous wall,

analogous to the sinus wall, should constitute a mechanical filter, altho the filtration process here taking place, has not been investigated.

There are pathological modifications of the sinus wall structure. The normal one can be designated as built up of a rectangular lattice-network. The fetal spleen demonstrates a deviation from this arrangement, as already indicated by BILLROTH. The reticular layer consists namely here of an irregular network containing circular meshes. The same structure is, strange enough, found in spleens from cases of chronic leukemias. Here one can hardly recognize any traces of the normal annular fibrils. In liver cirrhosis and chronic cardiac congestion the splenic wall has, on the other hand, a different appearance. Here one meets — as earlier pointed out by MATSUI — with an increasing number of longitudinal anastomoses between the annular fibrils. These anastomoses complete, in a way, the rod-cell layer, which results in a tightening of the sinus filter. Since the presence of a portal hypertension may be suggested in these cases, as leading up to an obstacle for the emptying of the sinuses and producing, consequently, a chronic sinus dilatation, the modification of the structure seems to secure a preservation of the tightness of the sinus filter.

The physiologic significance of the sinus filter seems to be, as previously mentioned, a filtration of the blood in such a manner that the red cells are retained within the sinuses while blood fluid and smaller particles are allowed to escape into the pulp cords. The modification that can be demonstrated under certain conditions indicates that the tightness of the sinus filter is, in some way, essential. Furthermore, we know that the deplasmatization of red cells, which is the filtration result of the blood suspension, brings about qualitative alterations in them. It is, then, highly probable that the main function of the spleen sinus wall is to deplasmatize red corpuscles, and so to hemolyze them.

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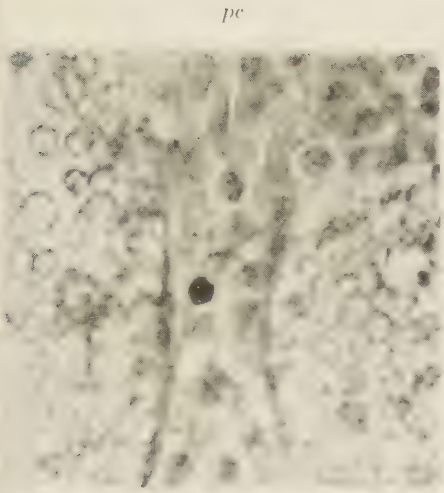

Fig. 2. Spleen from rabbit treated with gelatin before killing.
Almost bloodfree pulp cords. The dark spot is an iodine-stained starch granule. Magnific. × 800. (*pc* = pulp cord.) Kernechtrot.

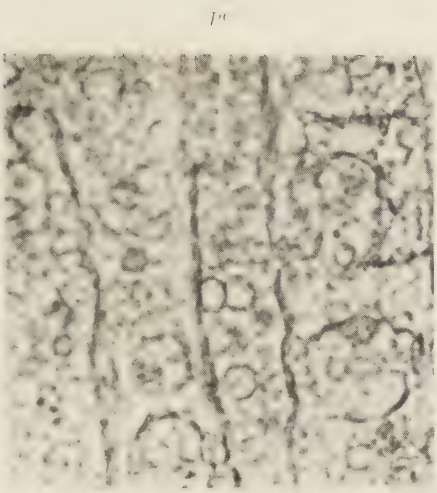

Fig. 3. Spleen from rabbit not treated before killing.
Pulp cords flooded with red cells. × 800. Kernechtrot.

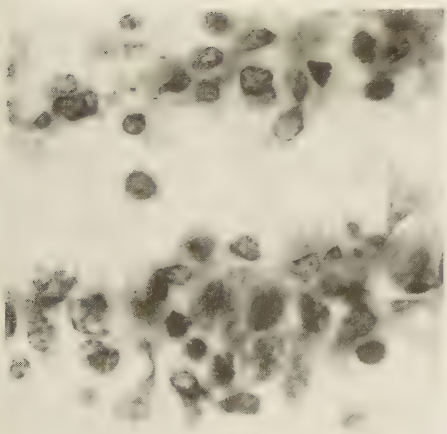

Fig. 4. Diapedesis of lymphocytes through sinus wall.
Normal rabbit. × 1000. Kernechtrot.

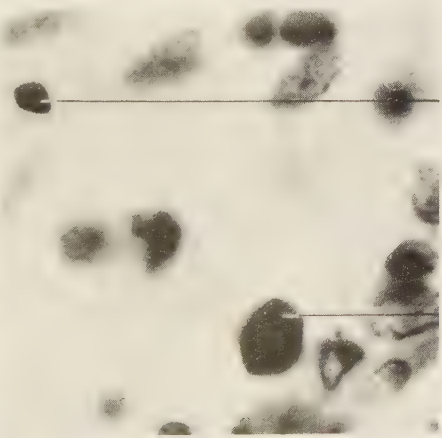

Fig. 5. Iodine-stained starch-grains in sinus and pulp cord.
× 1600. (*pg* = pulp-grain, *sg* = sinus-grain.) Kernechtrot.

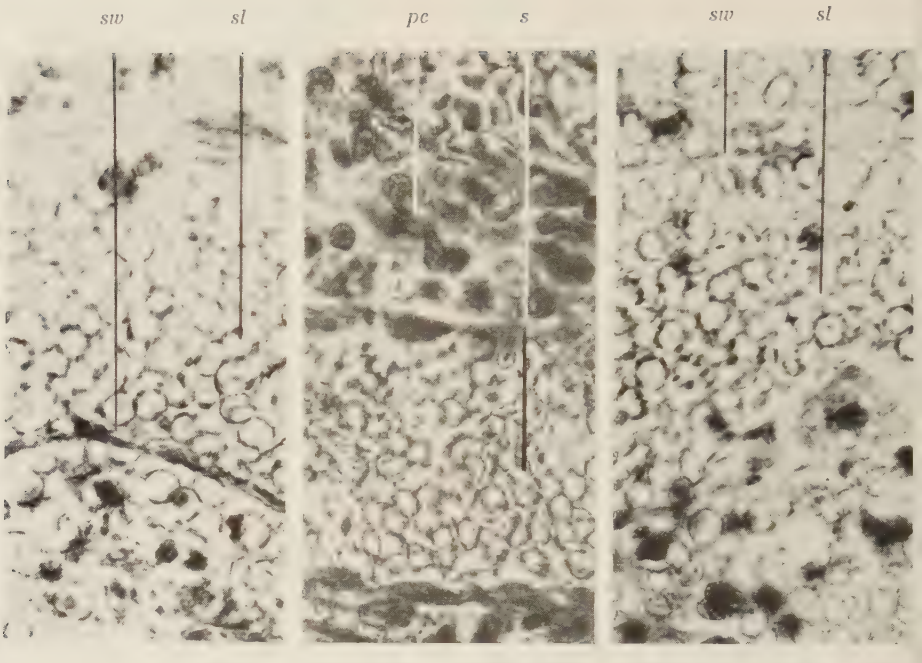

Fig. 6. Appearance of sinus and pulp cords in the different animal experimental groups. A. Infected group. B. Normal group. C. Poisoned group. Note the flooding of pulp cords in group A and C. (*sw* = sinus wall, *sl* = sinus lumen, *pc* = pulp cord.) × 800. Kernechtrot.

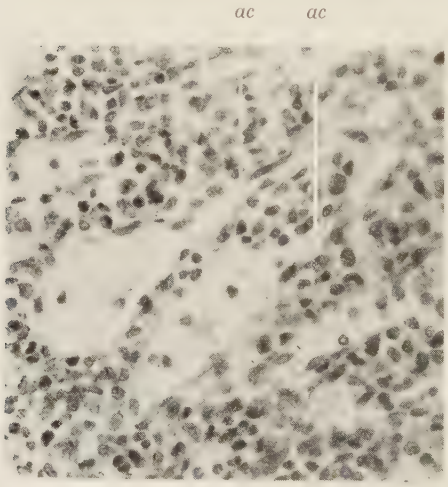

Fig. 7. Arterial capillaries opening into spleen sinuses.

Normal rabbit treated with gelatin. × 500. (*ac* = arterial capillary.) Kernechtrot.

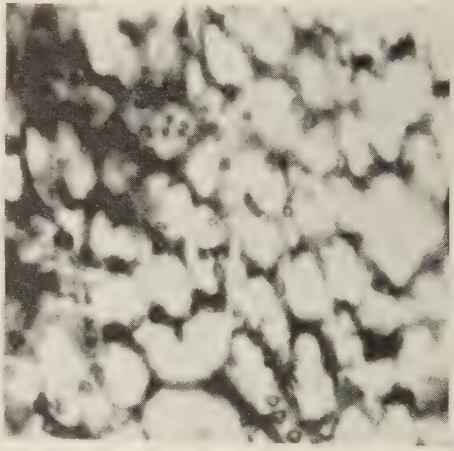

Fig. 8. Reticular network in the spleen sinus wall of rabbit.

× 1200. (The darker spots are silver-precipitations.) Snook.

Fig. 9. Appearance of the annular fibrils in Snook-impregnated sections.
A. Lattice-shaped network of ordinary contour. B. Lattice and dotted lines. × 600. Snook.

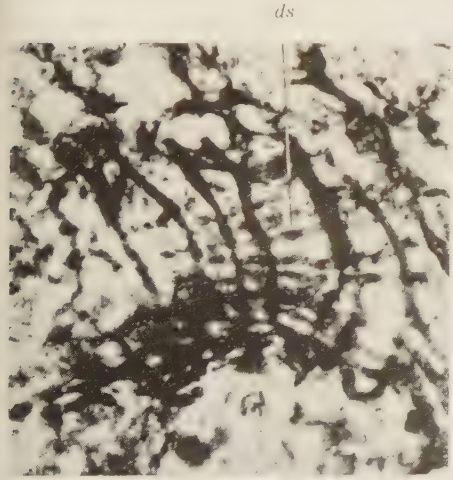

Fig. 10. Grid-like structure of sinus wall in human spleen.
× 1200. (*ds* = dark section.) Snook + Azan.

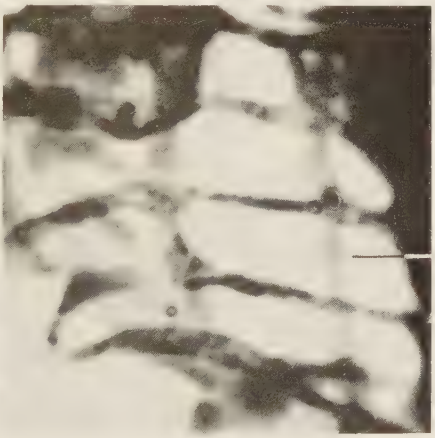

Fig. 11. Rectangular stoma in sinus wall of human spleen.
× 2500. (*ds* = dark section of rod-cell.) Snook.

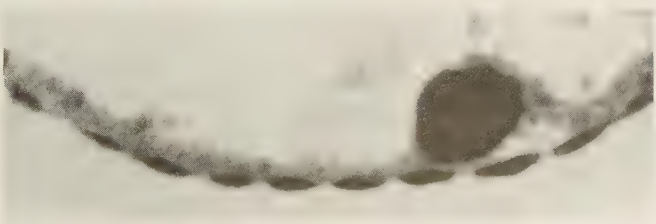

Fig. 12. Rod-cell from human spleen sinus wall.
Note the regular distances between the darker basal sections.
3000. Heidenhains iron hematoxylin.

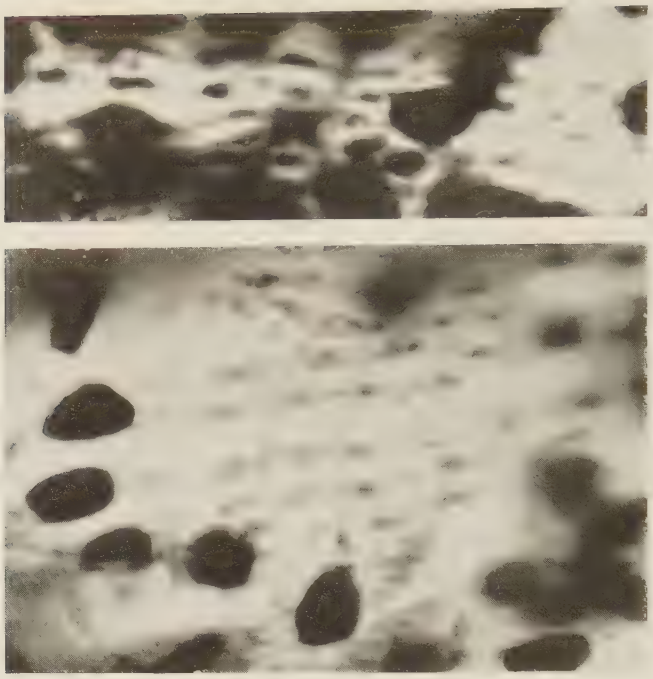

Fig. 13. Perfused human spleen.
A. Narrow sinus. B. Dilated sinus. Note the breadth of the darker spots, which are only slightly broadened in the dilated sinus. Their relative distances is on the average the same. 1600. Heidenheins iron hematoxylin.

Fig. 14. Sheathed capillary opening into a spleen-sinus in human spleen.
1200. (*cs* = capillary sheath, *sl* = sinus lumen.) Snook.

Fig. 15. Spiral screw of rod-cells at the sinus outlet. 1200. Snook – Wasserblau-orcein.

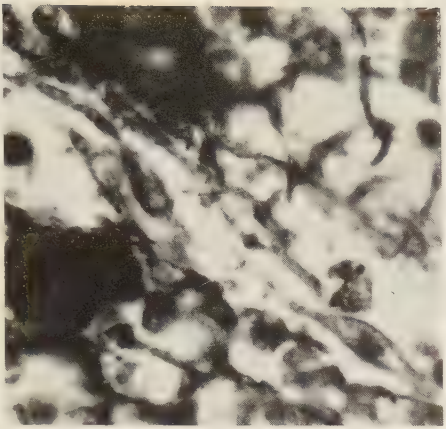

Fig. 16. Rod-cells crossing over to the opposite wall at sinus outlet. × 1200. Snook.

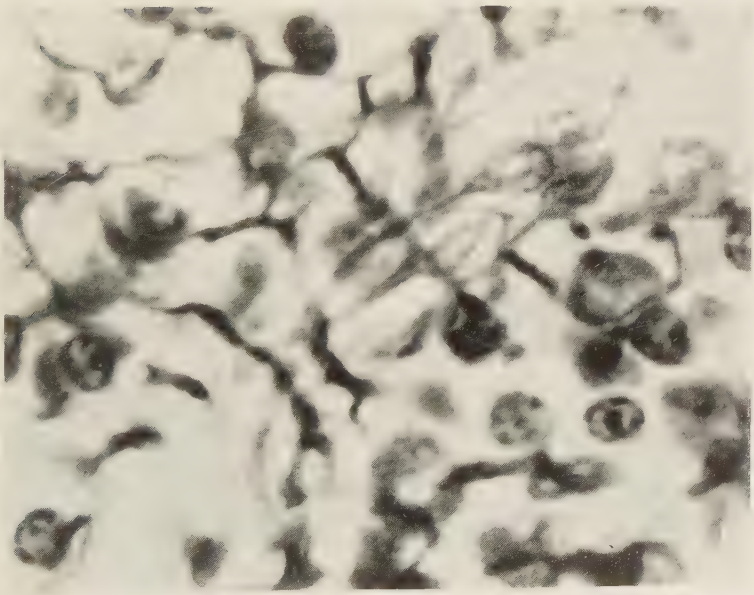

Fig. 17. Crossing rod-cells at the sinus venous orifice. 1600. Snook.

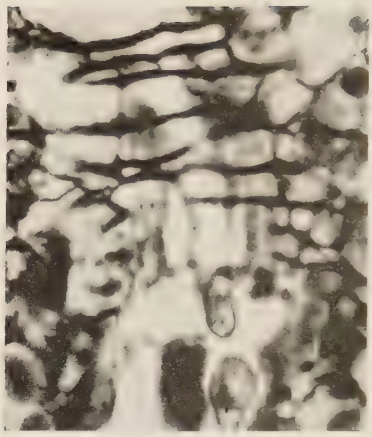

Fig. 18. Human spleen. Annular fibrils with anastomoses.
× 1000. Snook.

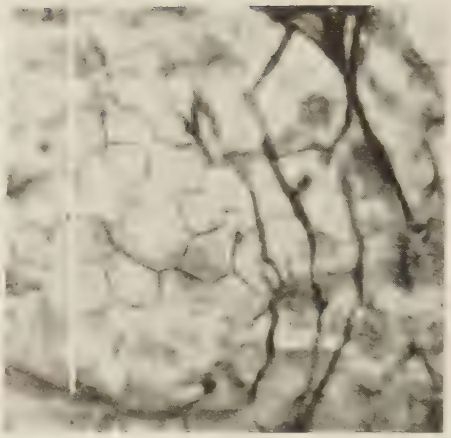

Fig. 19. Sinus crowded with red cells in case of septic splenitis in man.
× 1200. (*sw* = sinus wall.) Snook.

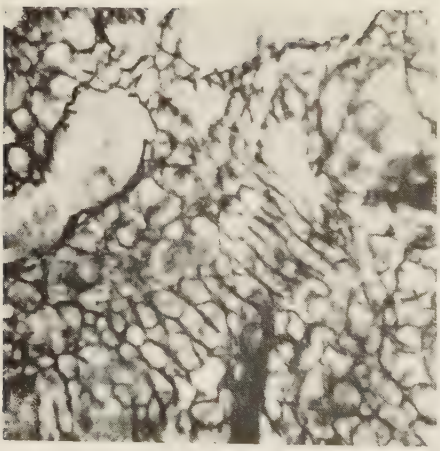

Fig. 20. Reticular network in fetal spleen.
× 600. Snook.

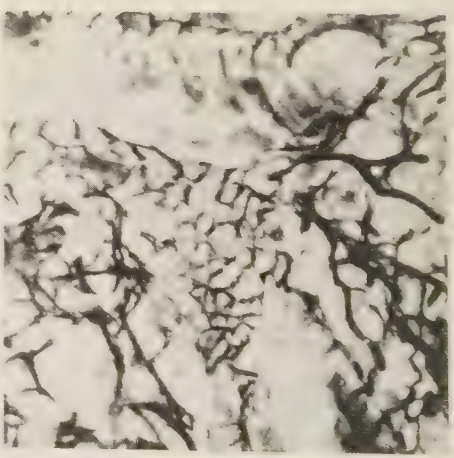

Fig. 21. Reticular network in adult spleen in chronic myeloid leukemia.
600. Snook.

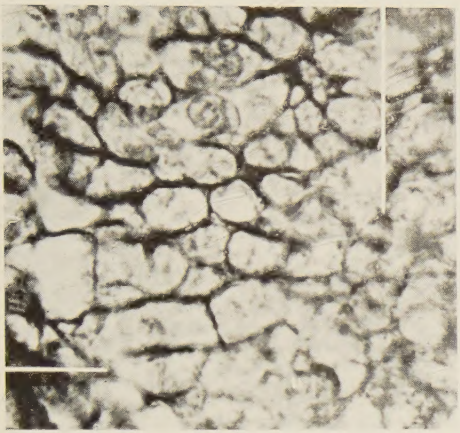

Fig. 22. Detail of sinus wall seen in Fig 20. Note the circular meshes. × 1500. (*sw* = end of sinus wall.) Snook.

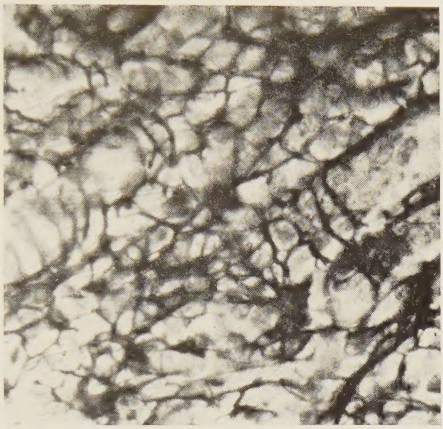

Fig. 23. Reticular sinus wall in chronic myeloid leukemia. × 800. Snook.

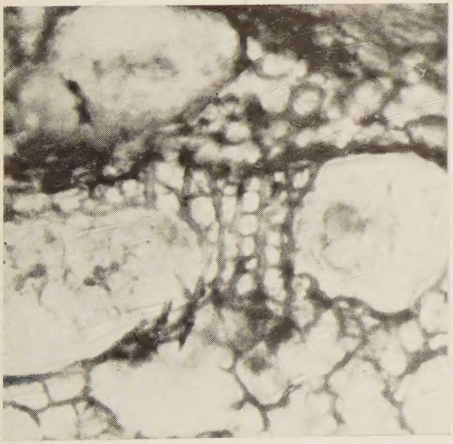

Fig. 24. Spleen sinus wall in liver cirrhosis. Note the fine longitudinal reticular anastomoses. × 1000. Snook.

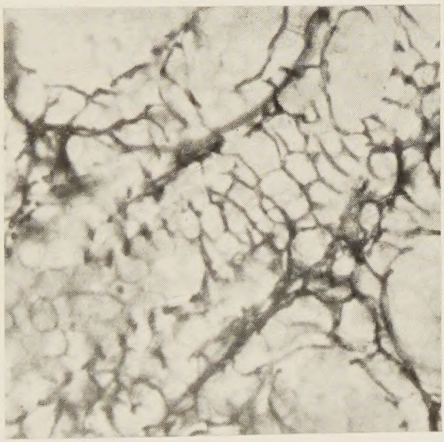

Fig. 25. Spleen sinus wall in chronic cardiac congestion. Longitudinal anastomoses but more scattered than in fig. 24. × 1000. Snook.

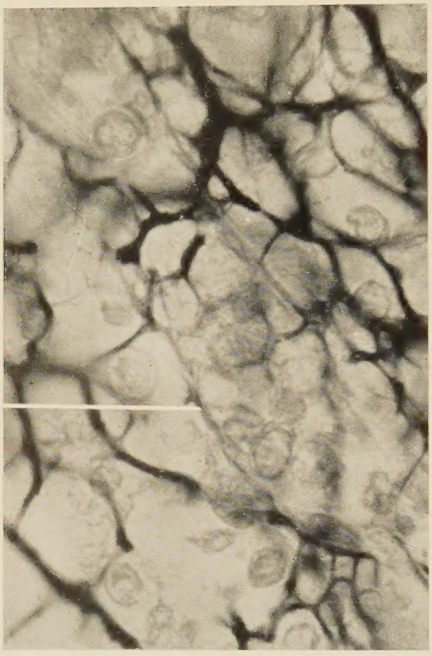

Fig. 26. Detail of sinus wall in chronic myeloid leukemia.

Note the circular meshes. × 1500. (*sw* = sinus wall.) Snook.

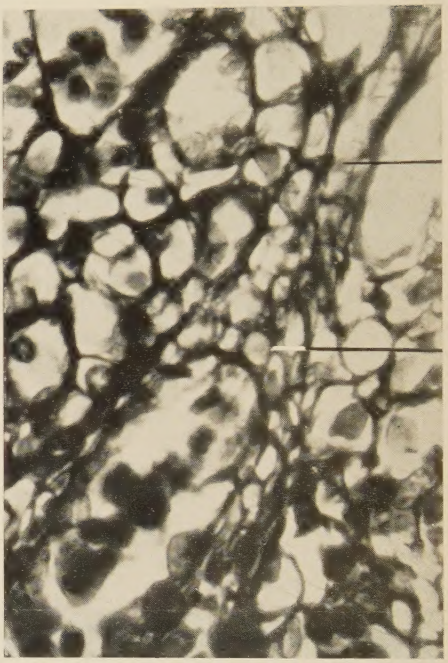

Fig. 27. Capillary opening into spleen sinus in chronic myeloid leukemia.

× 1000. (*c* = arterial capillary, *swm* = sinus wall meshes.) Snook.